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STUDIES ON CERCARIAL DERMATITIS AND THE TREMATODE FAMILY SCHISTOSOMATIDAE IN MANITOBA[1]

BY J. A. MCLEOD[2]

Abstract

A biological investigation of cercarial dermatitis in Manitoba has revealed that three species of apharyngeal brevifurcate cercariae are responsible for the disease. An ecological study of the organisms and their molluscan hosts has been carried out, and descriptions of the general types of habitats are given in addition to the distribution of the organisms. A synopsis of the dermatitis-provoking cercariae and the adult schistosomes of the world is given, and the classification of the adults is revised in part. The results of a survey of mammals and birds for adult schistosomes and also the results of experimental exposures of animals are included. A new species of apharyngeal brevifurcate cercaria and two new species of *Ornithobilharzia* are described. The description of *Cercaria wardlei* McLeod, 1934, is revised.

Introduction

An investigation of the etiology of swimmer's itch was undertaken in Manitoba in 1933, and the work has been carried on periodically since that time. Prior to the beginning of the work reported for the first time herein, it had been found that this local transitory skin disease of man was identical with the so-called cercarial or schistosome dermatitis in parts of the United States and various other countries. It had also been found that the disease in this province is provoked by the penetration of the skin by one or more species of brevifurcate larval trematodes or cercariae; that the organisms and their snail hosts are abundant in Clear Lake; that three species of adult schistosomes occur locally in wild ducks and a fourth species occurred in Herring Gulls collected near Yarmouth, Nova Scotia; also that a number of cercariae had failed to penetrate into, or reach maturity in, several species of experimental mammals and birds.

To elucidate the biology of the organisms concerned and bring our understanding of them to a point where control measures of some value might be suggested, the work has been continued and expanded. The purpose of the problem reported on herein, therefore, has been:—

1. A survey of southern Manitoba for dermatitis-provoking cercariae and their molluscan hosts.

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2. A brief study of the ecological factors responsible for their abundance and distribution.
3. A continuation of the survey of birds and mammals in an effort to discover what adult schistosomes occur in this area.
4. Exposure of experimental mammals and birds to the cercariae in an attempt to complete the life-history stages.
5. A study of related but non-dermatitis-provoking cercariae.
6. A study of the literature on larval and adult schistosomes of the world to determine the relationships of the local forms.
7. A revision of the classification of the Schistosomatidae.

Historical

The phylogeny of the schistosomes is not clearly understood at the present time, but the parasitic habit of life among them is apparently one of long standing. That the adults of one species have been the inhabitants of the blood vessels of man for at least 30 centuries has been proved beyond doubt by Ruffer (129). The first species of schistosome was discovered by Rudolphi in 1819 and was of the so-called non-human type, being a parasite of birds. *Schistosoma haematobium*, the second species recorded, was a true parasite of man and was discovered by Bilharz in Egypt in 1852. It was first recorded as *Distoma haematobium* but received its present name from Weinland in 1858. The second schistosome of man, *S. japonicum*, was described by Katsurada in 1904, and a third, *S. mansoni*, also well known at the present time, by Sambon in 1907.

In the interval between 1819 and 1912 thirteen species of schistosomes were recorded, but at that time nothing was known regarding the life-histories of the organisms or their method of gaining entrance to the host. It may be noted, however, that in 1896 Looss suggested it might be by active penetration of the skin.

The species recorded during this interval and the type of host in which each was found are as follows:—

Ornithobilharzia canaliculata (Rudolphi, 1819) Odhner, 1912, from birds.
Schistosoma haematobium (Bilharz, 1852) Weinland, 1858, from man.
Schistosoma bovis (Sonsino, 1876) Blanchard, 1895, from mammals and man (?).
Bilharziella polonica (Kowalewski, 1895) Looss, 1899, from birds.
Ornithobilharzia kowalewskii (Parona and Ariola, 1896) Odhner, 1912, from birds.
Dendritobilharzia pulverulenta (Braun, 1901) Skrjabin, 1924, from birds.
Schistosoma japonicum Katsurada, 1904 (S. cattoi Blanchard, 1905), from man.
Schistosoma indicum Montgomery, 1906, from mammals.
Schistosoma spindalis Montgomery, 1906, from mammals, rarely man.
Ornithobilharzia bomfordi (Montgomery, 1906) Price, 1929, from mammals.
Schistosoma mansoni Sambon, 1907, from man.

Gigantobilharzia acotylea Odhner, 1910, from birds.

Ornithobilharzia intermedia Odhner, 1910, from birds.

In the years 1913–14, Miyairi and Suzuki demonstrated that in the case of *S. japonicum* a parthenogenetic stage is passed through in a molluscan host and that the cercarial larvae so produced gain entrance to the appropriate warm-blooded host by active penetration of the body surface. This cleared the way for further work and the chronology of events from then up to the present time is as follows:—

Schistosoma japonicum, life-history by Miyairi and Suzuki, 1913.

Ornithobilharzia turkestanicum (Skrjabin, 1913) Price, 1929, from birds.

Schistosoma haematobium and *S. mansoni*, life-histories by Leiper, 1915.

Austrobilharzia terrigalensis Johnston, 1917, from birds.

Schistosoma spindalis, life-history by Soparkar, 1918.

Trichobilharzia ocellata (Skrjabin and Zakharow, 1920) Brumpt, 1931, from birds.

Schistosomatium pathlocopticum Tanabe, 1923, from mammals, life-history by Tanabe, 1923.

Macrobilharzia macrobilharzia Travassos, 1923 (Price, 1929, 1931), from birds.

Ornithobilharzia odhneri Faust, 1924, from birds.

Dendritobilharzia loossi Skrjabin, 1924, from birds.

Schistosoma incognitum Chandler, 1926, from man.

Schistosoma bovis, life-history (?) by Faust, 1926.

Pseudobilharziella yokogawai (Oiso, 1927) Ejsmont, 1931, from birds. Life-history by Oiso, 1927.

Ornithobilharzia, 2 sp. Linton, 1928, from birds.

Schistosoma faradjei Walkiers, 1928, from man (?).

Schistosoma mattheii Le Roux, 1929, from mammals.

Macrobilharzia anhingae (Price, 1929) Price, 1931, from birds.

Heterobilharzia americana Price, 1929, from birds.

Microbilharzia chapini Price, 1929, from birds.

Pseudobilharziella kowalewskii Ejsmont, 1929, from birds.

Schistosomatium douthitti H. F. Price, 1929, from mammals.

Ornithobilharzia pricei Wetzel, 1930, from birds.

Schistosomatium douthitti, life-history by Price, 1931.

Schistosoma rodhaini Brumpt, 1931, from mammals.

Schistosoma cucassoni Brumpt, 1931, from mammals.

Trichobilharzia ocellata, life-history by Brumpt, 1931.

Bilharziella polonica, life-history by Brumpt, 1931.

Bilharziella margebowiei Le Roux, 1933, from mammals.

Schistosoma nasalis Rao, 1933, from mammals.

Schistosoma suis Rao, 1933, from mammals.

Schistosoma intercalatum Fischer, 1934, from mammals and man.

Microbilharzia manitobensis McLeod, 1936, from birds.
Microbilharzia canadensis McLeod, 1936, from birds.
Microbilharzia lari (Syn. *Ornithobilharzia lari*) McLeod, 1937, from birds.
Pseudobilharziella querquedulae McLeod, 1937, from birds.
Ornithobilharzia sp. Young, 1937, from birds.

Exclusive of the two species reported for the first time in this thesis, this makes a total of 25 species known to infest birds. Of these only three life-histories have been completed. There are also 18 species from man or other mammals, of which seven life-histories are known.

Fourteen of the above 43 species were first recorded from North America and an additional one, *Bilharziella polonica*, originally described from Poland, has since been found on this continent by Price (114). Creech and Miller (45) encountered cases of nasal granuloma in cattle in Louisiana, U.S.A., with symptoms identical to those occurring in India; but the causal organism, later described by Rao (124) as *S. nasalis*, was not recovered in America. The life-histories of *Schistosomatium pathlocopticum* and *Schistosomatium douthitti*, both originally described from America, are known. They spend their adult stages in rats and mice, Tanabe (149), H. F. Price (116). *S. douthitti* has also been found in wild muskrats (*Ondatra zibethica*) in Michigan and Minnesota by Penner (117).

The three common species of human schistosomes, undoubtedly, have been introduced many times into North America, but that they never have become established seems to be fairly certain. This may be due to existing sanitary conditions, absence of suitable molluscan hosts, or unfavourable climatic conditions.

Cercarial Dermatitis

In many countries including Canada and the United States, where native schistosomiasis is unknown or endemic, the occurrence of a transitory skin disease, popularly known as "swimmer's itch" or "cercarial dermatitis", has been known for many years. A true causal organism was first associated with it by Cort (36, 37) in Michigan, and the condition is now widely recognized to be the result of attacks by schistosome larvae. For some time the disease was thought to be confined to man, but a case in dogs has been reported recently by Herber (59). In addition, the writer has information to the effect that in earlier times on the western prairies, cattle wading in sloughs were frequently afflicted with a dermatitis on the legs and belly.*

Following the report of Cort's discovery, numerous authentic cases of cercarial dermatitis, that previously would have been attributed, no doubt, to attacks of chigoes, chiggers, larval hookworms, various insects, or put down to unknown causes, were reported. Christensen and Greene (26) reported cases from lakes in the vicinity of Minneapolis, Minnesota. Here the causal organism was found to be a schistosome larva whose adult stage is, even yet, unknown and which had been tentatively named *Cercaria elvae* by Miller (96, 97).

**Personal conversation with Mr. Stuart Criddle, Treesbank, Man.*

Matheson (88) reported an outbreak among bathers at an artificial lake at Cardiff, Wales. He considered a species of furcocercous cercaria to be the causal organism and identified it as *C. elvae*. Taylor and Baylis (151) examined similar material from the same lake a short time later and were able to produce a dermatitis in experimental subjects but identified the organism as *C. ocellata* La Val. St. George, 1855.

Szidat (147) reported a large number of cases from near Rossitten in East Prussia, in which he suspected the larva of *Bilharziella polonica;* but many experimental exposures of susceptible individuals failed to prove that this organism was a dermatitis provoker or could penetrate human skin. Vogel (153–155) observed a good many natural infections of what appeared to be cercarial dermatitis in bathers in the Holstein Lake region south of Kiel, Germany. He was successful in producing experimental infections in volunteers with *C. ocellata* from snails in the lakes where natural infections were known to have occurred. He made a careful study of the similarity of cercarial dermatitis in Germany and the skin eruptions in human beings reported by Nageli (109) from the Bodensee region in Switzerland.

It must be noted that none of these observers succeeded in identifying the adult phases of the parasites concerned. The work of Brumpt (17–19) in France, on material collected near Paris, not only confirmed the belief that *C. ocellata* was a dermatitis provoker but also that it was the larva of *Trichobilharzia ocellata* (Skrjabin and Zakharow, 1920) Brumpt, 1931. Price in 1931 (115) completed the life-history of *C. douthitti* Cort, 1916, which has since been found to provoke a dermatitis.

The etiology and epidemiology of cercarial dermatitis have been summarized in a number of articles the most valuable of which are by the following investigators: Fülleborn (58), Szidat and Wigand (148), La Rue (70), and Cort (38, 39). The disease was first reported from Canada by the writer in 1934 (92) and at that time it was thought to be confined to Clear Lake, Manitoba. However, information gathered personally during the past five years indicates that the causal organisms and their molluscan hosts are common in the majority of prairie lakes and sloughs in southern Manitoba (Fig. 2).

Dermatitis-provoking Cercariae

The number of species of larval trematodes that definitely bear the potentiality for provoking human dermatitis is uncertain at the present time. This is to be understood in view of the difficulty encountered in identifying species even by experienced workers. The larvae show very indefinite physiological characters and the tissues exhibit little differentiation. Cercariae from a single snail host show a fairly wide variation in size so that metromorphic characters are not to be relied upon entirely. No doubt, what are considered now as single species in a good many cases will be found later to represent a complex of several species.

Human susceptibility to the penetration of the organisms must also be taken into account. Apparently certain species of cercariae cannot penetrate the skins of all people and probably only a few species of those that do penetrate provoke a sensible skin reaction. This is particularly true of the schistosomes of man, concerning whose dermatitis-provoking powers there is a division of opinion.

A number of investigators have produced evidence both for and against the hypothesis that the cercariae of *S. haematobium*, *S. mansoni*, and *S. japonicum* are responsible for a dermatitis in man. A number of Japanese workers are of the opinion that "kabure", which resembles cercarial dermatitis in the United States, results from the attacks of the cercariae of *S. japonicum*. Miyagawa (100), however, maintains that this is not so, as "kabure" occurs in regions where schistosomiasis is not present and also that in endemic areas "kabure" does not result in infestations with adult *S. japonicum*. Faust and Meleney (55) state that a skin eruption does not occur among rice farmers in China where schistosomiasis is endemic, and Faust, Jones, and Hoffman (56) state that in Puerto Rico the cercariae of *S. mansoni* do produce a prickling sensation while penetrating the skin but a dermatitis does not result.

Vogel (155) found that the cercariae of *S. mansoni* produced a slight dermatitis on the arm of a white subject but not on two negroes exposed in the same manner. Manson-Bahr and Fairley (87) saw cases of dermatitis among Arabs in Egypt which definitely were due to attacks by the cercariae of *S. haematobium* and *S. mansoni*. Lee (71) saw a number of cases among gypsies in the Yangtse River area in China, and Bettencourt *et al.* (6) observed a case on the arm of a woman in Portugal. In each case the cercariae of human schistosomes were known to be present in the water with which the individuals had come in contact or were recovered from snails in the vicinity.

Fülleborn (58) is of the opinion that "kabure" results from the penetration of human skin by non-human schistosome cercariae. Cort (38, 39) is inclined to the view that the cercariae of the so-called human schistosomes seldom produce a significant dermatitis and that cercarial dermatitis is associated with the reaction of an abnormal host to the penetration of a non-specific cercaria. The writer is of the opinion that individual resistance and susceptibility are important factors as some exposures of human beings to non-human schistosome cercariae are negative, while positive cases vary greatly in extent and severity.

It is improbable that all the species of non-human schistosome cercariae can produce a dermatitis in man. The nature of the stimulus to which the organisms give a positive response is not well known and the morphological characters that determine certain aspects of their behaviour are poorly understood. It is not clear how many may be attracted to the human skin but be unable to penetrate it.

At present there are four general types of schistosome cercariae as regards tactic behaviour towards man:

1. Those that do not respond positively to the presence of a human being in the water.
2. Those that respond positively but are unable to penetrate the skin.
3. Those that respond positively and penetrate the skin but, owing to lack of balance between the host and parasite, do not advance deeply and thus provoke only a localized skin reaction.
4. Those that respond positively, penetrate the skin, and eventually make their way to the portal vein and its branches, where sexual maturity is reached. In this case it is thought that frequently no skin reaction occurs.

From the information available it can be assumed that only the cercariae of the eight schistosome species of man belong to group 4. A good many known species may belong to groups 1 or 2, but at the present time only eight species of non-human schistosome cercariae can be placed in group 3. Undoubtedly more will be added in the future, as a schistosome dermatitis is known in several localities where the causal organism has not been definitely identified, or erroneously identified as one of the eight so-called dermatitis provokers.

Those definitely known to provoke dermatitis are as follows:

Cercaria ocellata (La Val. St. George, 1855) Ssintzin, 1909.

C. douthitti Cort, 1916.
C. elvae Miller, 1923.
C. tuckerensis Miller, 1927.
C. stagnicolae Talbot, 1936.
C. physellae Talbot, 1936.
C. pseudoocellata Szidat, 1933.
C. dermolestes sp. nov.

In 1934 the writer incriminated two additional cercariae, both of which were described as new species. *C. bajkovi* McLeod, 1934, is an invalid species, as the characters of two new species were confounded and the description corresponds with neither one. Both species have since been described by Cort and Brackett (42, 43). Similarly the unnoticed production of small numbers of *C. stagnicolae* simultaneously with *C. wardlei* led the writer to assume that the latter was a dermatitis provoker. This work has been rechecked but requires additional confirmation one way or the other.

Furcocercous Cercariae of Manitoba

Seven species of longifurcate pharyngeal cercariae have been recognized in Manitoba, but repeated experiments have proved fairly conclusively that none of them is capable of provoking dermatitis in man. They are as follows:

Species	Manitoba host
Cercaria of *Diplostomum flexicaudum* Van Haitsma, 1931 (*C. flexicauda* Cort and Brooks, 1928)	*Lymnaea stagnalis jugularis* *Stagnicola emarginata canadensis*
Cercaria of *Cotylurus flabellifirmis* Van Haitsma, 1931 (*C. douglasi* Cort, 1917)	*S. emarginata canadensis* *S. palustris elodes*

C. yogena Cort and Brackett, 1937	*S. emarginata canadensis* *S. palustris elodes*
C. dohema Cort and Brackett, 1937	*L. stagnalis jugularis* *S. emarginata canadensis*
Cercaria of *Crassiphiala ambloplitis* Hunter, 1937 (*C. bessiae* Cort and Brooks, 1928)	*Helisoma trivolvis*
C. burti Miller, 1923	*H. trivolvis*
C. wardlei McLeod, 1934	*S. emarginata canadensis*

In addition to the foregoing, three species of brevifurcate apharyngeal cercariae are common in Manitoba, and all three definitely are dermatitis provokers. They are as follows:

Species	Manitoba host
C. elvae Miller, 1923	*L. stagnalis jugularis*
C. stagnicolae Talbot, 1936	*S. emarginata canadensis*
C. dermolestes sp. nov.	*S. palustris elodes*

Descriptions of Cercariae

Cercaria dermolestes sp. nov.

Specific diagnosis: An apharyngeal brevifurcate cercaria with a prominent pair of pigmented eyespots. Entire body and tail uniformly spined. Body fusiform with the greatest diameter in the region of the acetabulum. Average measurements of specimens fixed in hot 10% formalin are as follows: Body 226μ in length by 70μ in maximum width; the anterior organ is 68μ in length and the acetabulum is 24μ in diameter and situated 94μ from the posterior end of the body. Tail stem 340μ by 42μ and furcae 210μ in length. Dorso-ventral furcal fin folds are present. Five pairs of penetration glands are present, two being granular and circum-acetabular in position, while the remaining three pairs are post-acetabular in position. These open at the anterior end just lateral to the mouth on the oral sucker by means of fine ducts, the openings of which are located on fine spines. A pair of small escape glands is present in specimens dissected from the sporocyst. Digestive system consists of a tubular oesophagus, which branches in the region of the eyespots into two short blind caeca. The excretory system is composed of seven pairs of flame cells and their ducts. In each lateral half there are three anterior to the acetabulum, three posterior to the acetabulum, and one in the tail stem.

Host: *Stagnicola palustris elodes* Say.

Locality: Southern Manitoba, Canada.

If the above organism constitutes a legitimate species, this makes a total of four species of cercariae that are identical except for minor metromorphic differences, slight differences in behaviour, and also differences in the choice of snail hosts. They are as follows: *C. elvae* Miller, 1923, *C. stagnicolae* Talbot, 1936, *C. physellae* Talbot, 1936, and *C. dermolestes* sp. nov. The former three were considered as being cospecific for several years but were separated by Talbot in 1936.

All four have the same time of emergence from the snail host, namely 4.30 to 8.00 a.m. According to Talbot (150) and Cort and Talbot (41), *C. elvae* is positively phototactic and in the resting position attaches itself to the wall of the container and assumes a characteristic shape. The body is arched and the tail is bent at an acute angle over the back in the form of a hook with the furcae always crossed.

C. stagnicolae, according to the same authors, is always positively phototactic and hangs suspended from the surface of the water in the region of the greatest light intensity. In this case the body and tail stem are extended and rigid and the furcae are widely separated. They do not, however, attach themselves to the sides of the container.

C. dermolestes sp. nov. is morphologically almost identical with the three species described by Talbot (150) and is of approximately the same size but differs somewhat in relative body measurements. It most closely resembles *C. stagnicolae* Talbot, but the body is shorter, being 226μ on the average, and the tail stem is longer, being 340μ. It resembles *C. stagnicolae* in its behaviour but is a slow sluggish swimmer, and the resting periods are relatively short. At this time it hangs straight down in the water with the furcae only moderately diverged. It has less tendency to bend the body ventrad, so that frequently dorso-ventral mounts can be obtained.

To the writer's knowledge no other schistosome type of cercaria has been recorded from *Stagnicola palustris* except *C. douthitti* and, in view of the fact that at least six species of adult schistosomes occur in Manitoba, this might reasonably belong to a separate species. Larger numbers of this organism can penetrate a thicker barrier of clothing than either of the two other local species and the resulting dermatitis is much more pronounced. Thus, on the basis of morphological and physiological characters, host specificity, and pathogenicity, the writer is of the opinion that the above represents a new species and the name *Cercaria dermolestes* is suggested.

Cercaria wardlei McLeod, 1934 (Fig. 1; Plate I, 3)

This species was recorded from Clear Lake in 1934, but the description, while correct in all major points, is incorrect in one minor point and is not sufficiently detailed to be of any great value in identification. For this reason it is considered advisable to revise and extend the description.

SPECIFIC DIAGNOSIS: A large longifurcate pharyngeal cercaria with a stout body which is pointed at the anterior end, but the sides are almost parallel. Body measurements vary greatly between the largest and the smallest specimens, but the average measurements of a wide range of material fixed in hot 10% formalin are as follows: Body 194μ long by 45μ wide. The anterior organ measures 39μ in length, and the acetabulum which is 41μ in diameter has a distance of 95μ between its anterior margin and the anterior end. The tail stem is 298μ in length by 32μ in width and the furcae are 295μ in length. The oral region is surrounded by a dense band of about six rows of spines, and in the circum-oral spineless area dorsal to the mouth is located a group of

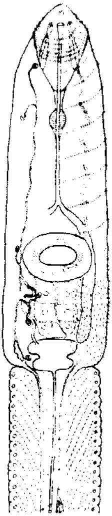


FIG. 1. *Semidiagrammatic sketch of Cercaria wardlei; body and anterior portion of tail stem. About × 600.*

twelve anteriorly directed spines. The pre-acetabular region bears nine definite rings of spines and the post-acetabular region is uniformly spined. The tail stem bears about ten pairs of long lateral filaments. The pharynx is prominent and the caecal branches reach almost to the posterior end of the body. Three pairs of penetration glands are located posterior to the acetabulum and their ducts open lateral to the mouth. The excretory system is made up of eight pairs of flame cells and their tubules. In each lateral half, two of each anterior group of three are located in the pharyngeal region and the third lies lateral to the acetabulum. The posterior group of three are directed mesad and are fairly uniformly spaced. Two pairs are present in the tail stem and are equally spaced in the second and third fourths. Each lateral collecting tubule has a distinct convolution near its anterior end, and in each arm of this there is a patch of long motile filaments. The excretory bladder is large and has a constriction near its middle, at which point contractions take place during the constant pulsations of the bladder. Caudal bodies are absent, but there are lateral rows of about fifty musculo-epithelial cells in the tail stem. They have distinct nuclei, and the inner muscular portion shows distinct transverse

striations. Such cells are absent or much less prominent in other local cercariae and form an easy and reliable means of identifying *C. wardlei*. A similar type of cell has been reported from cercariae by Ssintzin (139).

C. wardlei is a powerful swimmer, but the swimming periods are short and the organism rotates as it passes along. When agitated it will swim back and forth for a considerable time before becoming quiescent. The resting periods are long and the organism hangs body downward from the water surface with the long furcae widely separated. The pre-acetabular portion of the body may be flexed ventrad or the tail stem may be flexed near its anterior end throwing the body either to right or left.

HOST: *Stagnicola emarginata canadensis*.

LOCALITY: Clear Lake, Manitoba.

Molluscan Hosts in Manitoba

Manitoba lakes and sloughs may be divided into three general ecological groups as regards geographic position, temperature, type of bottom, vegetation, and hydrogen ion concentration. The first, or what may be classed as the oligotrophic type, occurs in the eastern and northern part of the province, involving the Manitoba portion of the Pre-Cambrian shield and commonly known as the eastern region (2). Here the lakes are numerous and are usually very deep with rocky bottoms and very little vegetation. The temperature is low throughout the summer, the oxygen concentration is high, and the pH is neutral or moderately acid. Snails are relatively scarce, and cercarial dermatitis has never been reported from this region, so that it has not been included to any extent in the surveys.

The second or eutrophic type includes the vast majority of Manitoba waters of the prairie region, such as lakes, ponds, and sloughs. Here the water is shallow, somewhat turbid, and the summer temperature is relatively high. The inwash of material is large, the bottom is of loose organic ooze varying in depth and overlying more solid gravel or clay. The pH is on the alkaline side of neutrality. The essential difference between the prairie lake and slough is one of size and also of the amount of vegetation present in the littoral region owing to the differences in water currents.

In the typical prairie slough, plant zonation of the littoral region is fairly distinct. Farthest from the shore may be found such submerged or floating plants as filamentous and other algae, *Elodea* sp., *Potamogeton* sp., *Chara*, *Nitella*, and *Utricularia*. Toward the end of the warm season these may become very dense and reach to the water surface. Emergent vegetation near the shore usually shows more or less definite rings of *Typha*, *Scirpus*, and *Phragmites*. Among the dense vegetation there is usually an abundance of rotifers, nematode worms, crustaceans, snails, and larval insects.

Bays and protected bits of shore line of the larger bodies of water such as Lake Winnipeg, Lake Manitoba, and Lake Dauphin, in general, show much the same fauna and flora as the common slough. In many cases a definite

shore has not yet been formed in these recent lakes, and the body of water shades off into typical marsh or muskeg (Fig. 2; Nos. 11, 12, 14, 20, 21).

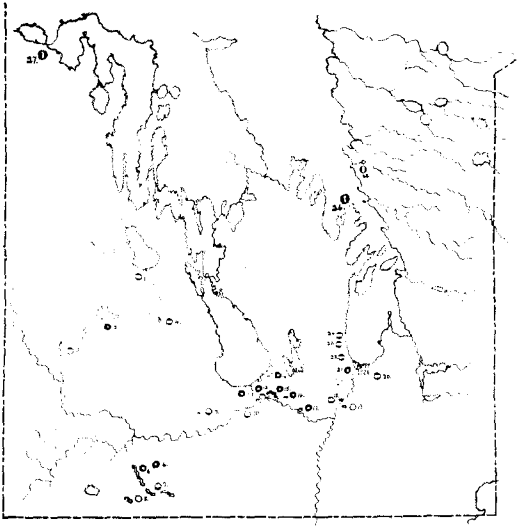


FIG. 2. *Map of southern Manitoba showing districts studied.* ◓ *Cercarial dermatitis known to occur.* ◒ *Molluscs found infested with schistosome cercariae and sporocysts.* ◑ *Districts from which birds have been examined.* ◐ *Districts from which birds were found infested with schistosomes.* 1. *Lake Dauphin,* 2. *Clear Lake,* 3. *Shoal Lake,* 4. *Gilmour Lake,* 5. *Pelican Lake, Ninette,* 6. *Pasture field slough near Ninette,* 7. *Lake Louise, Neelin,* 8. *Killarney Lake,* 9. *Artificial dam near Austin,* 10. *River lagoon, Portage la Prairie,* 11. *Delta marsh,* 12. *Portage Creek,* 13. *Black's Lake, High Bluff,* 14. *Lake Frances,* 15. *Long Lake, Raeburn,* 16. *Pasture field slough near Marquette,* 17. *Pigeon Lake,* 18. *River lagoon, St. Andrews,* 19. *Gravel pits, Birds Hill,* 20. *Libau marsh,* 21. *Netley marsh,* 22. *Whitewold,* 23. *Ponemah,* 24. *Gimli,* 25. *Catfish Creek,* 26. *Blackbear Island,* 27. *Mafeking.*

Clear Lake (Fig. 2; No. 2) represents a type intermediate between the oligotrophic and eutrophic lakes. It is situated at a relatively high altitude in an outcropping of the coniferous forest near the ecotone with the deciduous forest. There is little inwash of organic matter, as sizable inflowing streams are absent. The turbidity of the water is low and the currents are strong. The temperature is low, oxygen concentration high, and pH on the alkaline side of neutrality. The shore is rugged and cold water springs, either with or without definite channels, seep out of the bank in numerous places. The bottom near the shore is of fine sand and coarse stones or boulders. Emergent

vegetation such as reeds or rushes is sparse and confined to localized patches, but a growth of fine algae is found over the surface of the submerged stones.

Birds Hill area (Fig. 2; No. 19) represents an early stage of succession in rain and snow water pools in large gravel pits.

The molluscan fauna of Manitoba is moderately rich in species of Gastropoda, but many of these are few in numbers of individuals and are restricted in distribution (105, 106, 108). No doubt, in future more of these will be found to act as intermediate hosts to schistosomes, but only those that occur where cercarial dermatitis has been reported or suspected are included in the present paper. They are briefly as follows: *Lymnaea stagnalis jugularis* Say, *L. stagnalis perampla* Walker, *Stagnicola palustris elodes* Say, *Helisoma trivolvis* Say, *H. campanulata* Say, *Physella gyrina* Say, *Fossaria obrussa* Say, *Valvata tricarinata* Say, *Amnicola* sp.

L. stagnalis jugularis is one of the largest and most widely distributed snails of Manitoba. It occurs in practically all the prairie lakes and permanent sloughs where the summer temperature is high and the vegetation dense. It was obtained from all the districts studied (Fig. 2) with the exception of 3, 8, 13, 19, and 25, and in every case many were found to be infested with the sporocysts and were liberating cercariae of the dermatitis-provoking type.

S. palustris elodes is a smaller but extremely abundant form found coincidently with *L. stagnalis* in all districts except 9. In addition it was found in 19 and many other small or temporary bodies of water such as ditches, etc. Like *L. stagnalis*, wherever it occurs a sufficient distance from disturbance or human habitation, it is found to be the host to the larvae of schistosome and other types of trematodes.

S. emarginata canadensis, *L. stagnalis perampla*, and *F. obrussa* were found only at Clear Lake. *S. emarginata* is very abundant along the shore of almost the entire lake from a depth of 8 ft. to the water's edge. It is also the common host to the larval stages of a schistosome. Only three specimens of *L. stagnalis perampla* were found and these were uninfested. *F. obrussa* occurs in small numbers in the seepage water from springs around the edge of the lake. Only a few of these were examined, but all were free from trematode infestations. *H. campanulata*, *V. tricarinata*, and *Amnicola* sp. occur in the deeper waters of Clear Lake, but all three species were parasite free.

H. trivolvis and *Physella gyrina* occur in a wide range of situations but both were uninfested with schistosomes. On one occasion specimens of *H. trivolvis* from Pelican Lake were found to be infested with *C. burti* Miller and the cercariae of *Crassiphiala ambloplites* Hunter. An unidentified cercaria, possibly *C. multicellulata* Miller, was obtained on two occasions from *P. gyrina* at Clear Lake.

Primary Hosts in Manitoba

Many species of mammals are found in the vicinity of Manitoba lakes and sloughs and any one or more of these might reasonably act as the primary host of schistosomes. In recent years parasitological surveys of 460 Snowshoe

Rabbits (*Lepus americanus*) and 240 gophers (*Citellus*, 3 sp.) have been carried out in the laboratories of the University of Manitoba (12, 91) but all were negative for schistosomes. Other specimens examined included 36 muskrats (*Ondatra zibethica*) from Clear Lake and Netley and Delta marshes.

Representatives of the majority of Manitoba wild mammals have been examined in the laboratories of the Provincial Game and Fisheries Branch during the past eight years by Dr. J. A. Allan, Pathologist, but as yet no schistosomes have been found.

Five laboratory mice and three kittens were exposed to schistosome cercariae, but post-mortem examination one month later showed them to be free from blood trematodes.

Manitoba is visited annually by large numbers of water fowl either as migrants or summer residents. The ordinary prairie marsh or slough is the feeding and breeding ground of a good many ducks, coots, and grebes, as well as of a number of shore birds and waders. Gulls and terns are fairly common around the lakes and rivers, breeding in either neighbouring marshes or on rocky islands. Loons and merganser ducks nest around some of the deeper lakes but never occur in great numbers. Migrating cormorants, pelicans, and geese, as well as a number of others, remain for varying lengths of time in the spring and fall.

The fact that schistosome cercariae are widespread in Manitoba and that their occurrence coincides with that of the snail host except in certain small areas close to human habitations, indicated that the definitive hosts quite probably would be water birds. This condition, coupled with the fact that over half the known species of schistosomes have water birds as definitive hosts, led to the examination of all possible local species. However, insufficient numbers of some species were examined to give significant results.

Microbilharzia lari was recovered from Herring and Ring-billed Gulls collected at Lake Winnipeg and Clear Lake. Other specimens of Herring and Ring-billed Gulls from the same points as well as other places on Lake Winnipeg, Lake Winnipegosis, and Clear Lake yielded two additional species of schistosomes, namely: *Ornithobilharzia aviani* sp. nov. and *Ornithobilharzia filamenta* sp. nov.

Both species of gulls occur in considerable numbers around the larger Manitoba lakes and their tributaries. They also occur in moderate numbers at Clear Lake. A few loons and merganser ducks are found at Clear Lake, but ordinary pond or diving ducks are scarce during the breeding season or late summer. Blue-winged Teal and Golden-eye Ducks frequently alight and feed for a short time near the water's edge in the early morning.

The Blue-winged Teal is the commonest duck nesting around prairie lakes and sloughs. They are very numerous and widely distributed; over 60% of all specimens, both adult and juvenile, were infested with the males of *Pseudobilharziella querquedulae* McLeod, 1937. These were collected from a good many localities, and infested birds were encountered in every case regardless of the season.

TABLE I

BIRDS EXAMINED FOR SCHISTOSOMES

Specific name	Common name	No. examined	No. infested
Larus pipixcan	Franklin's Gull	7	0
Larus argentatus	Herring Gull	32	7
Larus delawarensis	Ring-billed Gull	20	5
Sterna hirudo	Common Tern	6	0
Dafila acuta	Pintail Duck	12	0
Querquedula discors	Blue-winged Teal	38	22
Nettion carolinense	Green-winged Teal	4	0
Anas platyrhynchos	Mallard Duck	9	0
Nyroca valisneria	Canvas-back Duck	3	1
Spatula clypeata	Spoonbill Duck	12	0
Mareca americana	Baldpate Duck	1	0
Nyroca marila	Bluebill Duck	3	0
Nyroca americana	Redhead Duck	30	0
Nyroca collaris	Ringneck Duck	1	0
Erismatura jamaiciensis	Ruddy Duck	2	0
Glaucionetta clangula	Golden-eye Duck	1	0
Branta canadensis	Canada Goose	1	0
Fulica americana	Coot	7	0
Colymbus nigricollis	Eared Grebe	3	0
Limosa fedoa	Marbled Godwit	1	0
Phalacrocorax auritus	Crested Cormorant	1	0
Bartramia longicauda	Upland Plover	1	0
Gavia stellata	Red-throated Loon	4	0
Gavia immer	Northern Loon	1	0
Ceryle alcyon	Belted Kingfisher	1	0
Botaurus lentignosus	American Bittern	1	0
Mergus americanus	American Merganser	4	0
Pelecanus erythrorhynchus	White Pelican	1	0

Total number of species 28. Total number of specimens 207.

The Canvas-back Duck is common in southern Manitoba only during the seasonal migrations. A single specimen infested with *Microbilharzia manitobensis* McLeod, 1936, and *Microbilharzia canadensis* McLeod, 1936, was collected in late fall at Lake Frances.

An attempt was made to infect several species of wild ducks experimentally with each of the three species of schistosome cercariae. Hatchery-reared birds of from one to six weeks of age were obtained and some of each species were exposed repeatedly in the laboratory for intervals of from 15 to 45 min. to water containing the cercariae. In other cases thick infusions of cercariae were injected intravenously, intraperitoneally, or subcutaneously by means of a hypodermic needle.

Birds Exposed Experimentally

Specific name	Common name	Number
Anas platyrhynchos	Mallard Duck	5
Dafila acuta	Pintail Duck	5
Nyroca valisneria	Canvas-back Duck	5
Nyroca americana	Redhead Duck	4
Querquedula discors	Blue-winged Teal	10
Anas boschas domestica	Domestic Duck	6

Control birds were kept in each case in order to check the accuracy of the experiment. One domestic duck exposed to *C. stagnicolae* developed a severe dermatitis on the second day and died on the sixth day as a result. Post-mortem examinations were carried out up to six weeks after exposure but all were negative.

Classification of the Schistosomatidae

The discovery of several new species of schistosomes in the past few years, particularly those recorded by Ejsmont (47), Wetzel (158), and the writer (93, 94), necessitates a revision of the classification and also the keys for the identification of the various members of the family. In the following keys Price's classification (114) is used as a basis, but a number of minor changes are suggested.

Price (115, 116) suggests the re-establishment of the genus *Macrobilharzia* Travassos, 1923, to accommodate *Paraschistosomatium anhingae* Price, 1929, and *Ornithobilharzia macrobilharzia* (Travassos, 1923) Price, 1929, and suggests that the genus *Paraschistosomatium* Price, 1929, be deleted. This suggestion has been adopted in the following classification.

The genus *Ornithobilharzia* Odhner, 1912, should be amended, as suggested by Wetzel (158), to include forms in which the female is shorter or longer than the male, having 28 to 120 testes in the male, and also having the spiral ovary of the female confined to the anterior one-third of the body. This would necessitate the amending of the genus *Microbilharzia* as follows: Suckers present in the male, present or absent in the female. Testes 18 to 26 in number in the male, ovary pre-equatorial in the female and in the form of a loose spiral. The species *Ornithobilharzia lari* McLeod, 1937, would then be transferred to the genus *Microbilharzia*. The creation of the new genus *Pseudobilharziella* by Ejsmont (48) calls for a revision of the generic diagnosis of the old genus *Bilharziella*, and he suggests the following changes: Anterior region narrow and one-half of the body length; posterior region broad and lanceolate. Genus *Pseudobilharziella:* Anterior portion of the body broad and one-fifth of the body length; posterior portion narrow and strap-like.

A modification of Price's classification in accordance with the above suggestions consequently would be as follows:

Key to the Sub-families of the SCHISTOSOMATIDAE

Females slender, more or less cylindrical in cross-section; males larger than females, flattened, and with the lateral edges infolded forming a gynaecophoric canal; intestinal caeca usually unite caudad of the equator of the body; testes situated cephalad of the caecal union . . . SCHISTOSOMINAE.

Females similar to males in shape; males without well developed gynaecophoric canal; caecal branches unite cephalad of the equator of the body; testes situated caudad of the caecal union. . . . . BILHARZIELLINAE.

KEY TO THE GENERA OF SCHISTOSOMINAE Stiles and Hassal, 1898

1. Females unknown; male gynaecophoric canal well developed; testes 70 to 83 in number, in posterior third of body and cephalad of the caecal union; in mammals. . . . . *Heterobilharzia*.

   Males and females both known. . . . . 2.

2. Testes 230 to 250 in number, from just caudad of acetabulum into posterior half of body; female slender, much shorter than male; ovary spiral, in the posterior third of the body; intestinal caeca unite in posterior end of body; uterus long and filled with eggs. . . . . *Macrobilharzia*.

   Gynaecophoric canal well developed; testes less than 120. . . . . 3.

3. Testes 28 to 120 in number, in the anterior two-thirds of the body; female shorter or longer than male; ovary spiral in anterior one-third of body . . . . *Ornithobilharzia*.

   Testes 26 or less in number; ovary just pre- or post-equatorial in position; suckers present in male, present or absent in female. . . . . 4.

4. Anterior end of gynaecophoric canal near equator of body; testes in two rows, at anterior end of gynaecophoric canal; genital pore immediately in front of anterior testis; intestinal caeca with short lateral diverticula; common caecum in both sexes short; ovary pre-equatorial. . . . . . *Schistosomatium*.

   Anterior end of gynaecophoric canal near acetabulum; genital pore of male a short distance caudad of acetabulum; intestinal caeca with diverticula reduced or absent; common caecum usually long; ovary pre- or post-equatorial in position. . . . . 5.

5. Testes less than ten in number; ovary oval. . . . . *Schistosoma*.

   Testes 18 to 26 in number; ovary spiral. . . . . 6.

6. Anterior end of gynaecophoric canal slightly caudad of acetabulum; oral sucker lacking in female; ovary about one-third of body length from the posterior extremity. . . . . *Austrobilharzia*.

   Anterior end of gynaecophoric canal cephalad of acetabulum; suckers present or absent in female; ovary pre-equatorial. . . . . . *Microbilharzia*.

KEY TO THE GENERA OF BILHARZIELLINAE Price, 1929

1. Body cylindrical or nearly so. . . . . 2.

   Body flattened. . . . . 3.

2. Female unknown; posterior end of body threadlike, middle portion wider than either the anterior or posterior portions; no gynaecophoric canal; suckers present. . . . . *Trichobilharzia*.

   Male and female very long and slender; gynaecophoric canal reduced to a short groove in the anterior part of the body; suckers absent . . . . *Gigantobilharzia*.

3. Suckers absent; common caecum with short lateral dendritic branches . . . . *Dendritobilharzia*.

   Suckers present; common caecum without lateral dendritic branches. . . . 4.

4. Body divided into narrow anterior and broad lanceolate posterior portions, the point of union being at the junction of the second and third fifths of the body . . . . *Bilharziella.*
   Body divided into broad anterior and narrow strap-like posterior portions, the point of union being at the junction of the first and second fourths of the body . . . . *Pseudobilharziella.*

KEY TO THE SPECIES OF THE GENUS MICROBILHARZIA Price, 1929

1. Testes in male reaching into post-equatorial region . . . . 2.
   Testes in male confined to pre-equatorial region . . . . 3.
2. Body fairly stout in male; caecal branches slightly convoluted and without diverticula; female unknown . . . . *M. chapini.*
   Body slender in male; caecal branches convoluted and with small diverticula; female unknown . . . . *M. manitobensis.*
3. Caecal branches in male uniting at about the junction of the middle and posterior thirds of body; common caecum long; acetabulum wanting in female . . . . *M. canadensis.*
   Caecal branches in male uniting near hinder end of body; common caecum short; acetabulum present in female . . . . *M. lari.*

ADULT SCHISTOSOMES OCCURRING IN MANITOBA

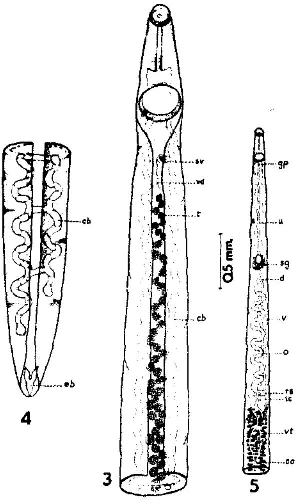
Species	Host
Microbilharzia canadensis McLeod, 1936.	*Nyroca valisneria.*
M. manitobensis McLeod, 1936.	*N. valisneria.*
M. lari McLeod, 1937.	*Larus argentatus.*
Pseudobilharziella querquedulae McL. 1937.	*Querquedula discors.*
Ornithobilharzia aviani sp. nov.	*L. argentatus.*
O. filamenta sp. nov.	*L. delawarensis.*
	L. argentatus.

Descriptions of New Species

Ornithobilharzia aviani sp. nov. (Figs. 3–5)

GENERIC DIAGNOSIS: *Ornithobilharzia.*

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter, reaching a maximum of 0.83 mm. The lateral edges of the body are infolded to form a well developed gynaecophoric canal which begins just caudad of the acetabulum and gradually becomes more pronounced. It is only moderately deep throughout most of its length and gradually disappears near the caudal end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed oblique ventrad. It is circular and has an average diameter of about 250 μ. The anterior body portion is elliptical in



FIGS. 3–5. *Ornithobilharzia aviani sp. nov.* FIG. 3. *Anterior portion of male.* FIG. 4. *Posterior end of male.* FIG. 5. *Anterior portion of female.* cb. *caecal branch;* cc. *common caecum;* d. *vitelline duct;* eb. *excretory bladder;* gp. *genital pore;* lc. *Laurer's canal;* o. *ovary;* rs. *receptaculum seminis;* sg. *shell gland;* sv. *seminal vesicle;* t. *testes;* u. *uterus;* v. *vagina;* vd. *vas deferens;* vt. *vitellaria.*

cross-section and the acetabulum is pedunculate. Its anterior margin is 0.82 mm. from the anterior end of the body and the average diameter is 400μ. The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back, it passes through a mass of tissue, partly muscular, partly glandular in nature. Cephalad of the acetabulum, it passes into a squarish cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a slightly convoluted course caudad. Farther back, the caeca become progressively larger and more convoluted and later, anastomosis occurs. There are some individual variations near the posterior end where there may be one or two anastomosing branches. Following this, the caeca may be of unequal length and end singly or may unite, the common caecum ending close to the posterior end of the body. A small but distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in a row, which at the anterior end is convoluted, while toward the hinder part it is made up of a compact mass about two testes in width. The row begins about 0.68 mm. caudad of the acetabulum and extends into the post-equatorial region. In mature speci-

mens, the testes are spherical, about 95μ in diameter, and vary from 54 to 74 in number. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. caudad of the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Filamentous worms considerably shorter than the males and usually completely enclosed in the gynaecophoric canal. The average length in fixed specimens is 7.2 mm. by 0.23 mm. in width. The portion anterior to the ovary is cylindrical and finely attenuated, while the hinder portion is flattened and bluntly pointed. The cuticle is finely tuberculate. The oral sucker is terminal and has an average diameter of 50 μ. The acetabulum is well developed and pedunculate, with a diameter of 63 μ. The digestive canal branches into two fine caeca just cephalad of the acetabulum. These pass caudad to just behind the ovary where they may unite permanently or separate and reunite once or twice. The common caecum follows a straight course to near the hinder end of the body. The ovary is a spiral consisting of about eight loops and begins 1 mm. from the acetabulum. Its length, disregarding the total length of the spiral, is 0.78 mm. A small receptaculum seminis is present at the posterior end of the ovary and Laurer's canal is also present. An oviduct of moderate size passes forward to the ootype, which is distinct and located 0.81 mm. caudad of the acetabulum. The vitellaria are in the form of numerous small oval follicles, which occupy most of the region caudad of the ovary. The vitelline duct passes forward in company with the oviduct to the ootype. The uterus is narrow, moderately straight and the genital pore opens behind the acetabulum. Eggs are produced singly.

CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, birds *(Larus argentatus)*, secondary, unknown.

TYPE LOCALITY: Lake Winnipeg, Manitoba, Canada.

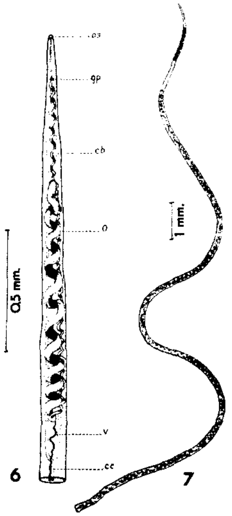
LOCATION: Portal and intestinal veins.

The above description is based on the examination of a few pairs of males and females in a good state of preservation, taken in copula from the intestinal veins of an adult specimen of *Larus argentatus*. Both males and females closely resemble those of *O. canaliculata* (Rudolphi, 1819) Odhner, 1912. The male also resembles that of *O. kowalewskii* (Parona and Ariola, 1896) Odhner, 1912, in so far as the brief descriptions of these two species go. Dr. Price suggests that there is a strong possibility of the two being cospecific, but because of the brevity of the published descriptions, it is impossible to establish the point. The writer considers it advisable to regard the above as a new species until such time as specimens of both *O. canaliculata* and *O. kowalewskii* can be examined in detail and the differences of similarities determined for all three.

Ornithobilharzia filamenta sp. nov. (Figs. 6 and 7)

GENERIC DIAGNOSIS: *Ornithobilharzia.*

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter reaching a maximum of 0.63 mm. The lateral edges of the body from just behind the acetabulum to very near the posterior end are infolded to form a fairly well developed gynaecophoric canal. It is only moderately deep in the mid-body region and becomes



FIGS. 6 and 7. *Ornithobilharzia filamenta sp. nov.* FIG. 6. *Anterior portion of female.* FIG. 7. *Entire female.* cb. *caecal branch;* cc. *common caecum;* gp. *genital pore;* o. *ovary;* os. *oral sucker;* v. *vitelline duct.*

progressively shallower toward the posterior end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed obliquely ventrad. It has a length of about 210 μ and a width of about 205 μ. The anterior portion of the body is elliptical in cross-section and the acetabulum is pedunculate. Its anterior margin is about 0.81 mm. from the anterior end and the average diameter is 330μ. The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back it passes through a mass of dense,

deeply staining tissue and anterior to the acetabulum it passes into a bulbous cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a convoluted course caudad. Toward the posterior end, they become progressively larger with traces of secondary branches and show a tendency to anastomose. In certain cases they unite to form a common caecum, but in others they unite by cross branches and each ends blindly. A small distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in an irregular single row, which follows a zig-zag course. The row begins about 0.7 mm. behind the acetabulum and extends to within about 4.4 mm. of the posterior end. In mature specimens the testes are oval, 89 μ in diameter, and vary in number from 88 to 112 in different individuals. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. behind the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Extremely long and filamentous, with a length of 15 to 20 mm. and a maximum width of 0.1 mm. A short anterior portion of the body is cylindrical and finely attenuated, while the remainder is broader with parallel sides, flattened, and ends bluntly. The oral sucker is feebly developed and is 16 μ wide. It is conical in shape and the aperture is subterminal in position. An acetabulum is entirely lacking and the cuticle is finely spined. The ovary is an elongated tubular body forming a spiral of about 8 to 9 turns and lies in the middle of the anterior one-eighth of the body, beginning about 0.61 mm. from the anterior end. The vitellaria are in the form of a single row of numerous relatively large follicles, each of which is almost as wide as the body. They occupy the posterior seven-eighths of the body. The oviduct passes forward from the posterior end of the ovary, and the uterus appears to open a short distance from the anterior end. The ootype and Mehlis' gland were not discernible and eggs were not present in the female worms. The alimentary canal is simple, branching midway between the oral sucker and the ovary, and the caecal branches reunite just posterior to the ovary. The common caecum is long, reaching almost to the posterior end and following a zig-zag course.

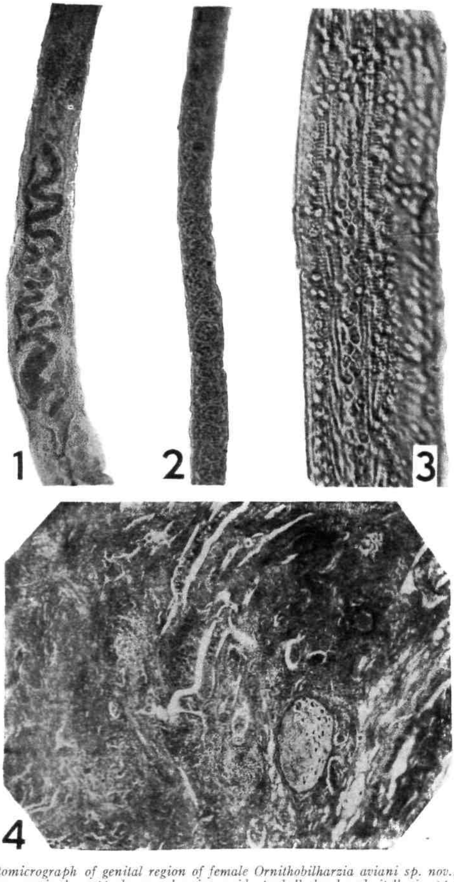
CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, Birds (*Larus delawarensis* and *L. argentatus*); secondary unknown.

LOCALITY: Lake Winnipeg and Clear Lake, Manitoba, Canada.

LOCATION: Coccygeomesenteric and mesenteric veins and lymphatics.

The male of *Ornithobilharzia filamenta* is almost identical morphologically with that of *O. aviani*, except for the number of testes present. The two species occur coincidently in the same host, so for this reason, only males actually in copula with the females have been used in determining the specific characters. The female differs greatly in size and also in the shape and position of the genitalia from that of *O. aviani*, and thus serves to distinguish the two



1. *Photomicrograph of genital region of female Ornithobilharzia aviani sp. nov., showing spiral ovary, seminal receptacle, caecal union, oviduct, shell gland and vitellaria,* × *60.*

2. *Photomicrograph of mid-body region of female Ornithobilharzia filamenta sp. nov. showing single row of large vitellaria,* × *60.*

3. *Photomicrograph showing tail stem musculature of Cercaria wardlei,* × *940.*

4. *Photomicrograph of section of lymphatic from infested Herring Gull showing sections of female Ornithobilharzia filamenta sp. nov. and fibrosed cyst containing what appears to be eggs,* × *20.*

species at a glance. *O. filamenta* has been found in the hepatic portal and mesenteric veins but appears to prefer the coccygeomesenteric vein, as females frequently are found there in large numbers. In some cases they are found to have migrated into the neighbouring lymphatics and produced granular, tumour-like swellings of considerable size. Histological sections of one of these tumours (Plate I, 4) show sections of several female worms and also what is taken to be groups of eggs that have been walled off by fibrous tissue. Lesions of the gut wall have not been found and free eggs have not been seen.

The males of the above described species, as in *O. aviani*, are very similar to those of *O. canaliculata* and *O. kowalewskii*. The female, in general, resembles that of *O. intermedia* and that of *O. pricei* but is much longer, the ovary is relatively farther forward, and the acetabulum is lacking entirely. The characters of the gynaecophoric canal and testes in the male and the ovary and vitellaria in the female definitely place the above organism in the genus *Ornithobilharzia*. It differs, as pointed out, particularly in the case of the female, from any described species and is submitted as being new to science. The name *Ornithobilharzia filamenta* is suggested.

Discussion

A total of 15 species of adult schistosomes has been recorded from the United States and Canada. One of these *(B. polonica)* had been described previously from other countries and the life-history worked out in detail, but the cercariae have not been reported from North America. Two of the 14 species considered as being native to North America have their adult stages in mammals (rodents), and in both species the life-history is known and the cercariae have been described and their distribution studied. The remaining 12 have been reported as adults from birds and the cercarial stages are either unknown or unrecognized. Whether the infestations are acquired in these two countries or in some other is, as yet, undetermined. Six distinct species of schistosome cercariae are thought to occur in North America, and no doubt several more will be discovered in the near future or are being mistaken at the present time for one or more of the recorded species.

Six species of adult schistosomes have been found in Canada by the writer, and all these occur in migratory birds in the southern half of Manitoba. Two species are rare, having been found only once in the Canvas-back Duck, a bird that occurs here only as a migrant.

Pseudobilharziella querquedulae is the commonest schistosome found locally. It occurs in 60% of all the specimens of *Querquedula discors* examined, regardless of the season or the place from which the birds were collected. *P. querquedulae*, undoubtedly, is the adult of one of the three local dermatitis-provoking cercariae, as immature worms have been recovered from fledgling birds that were not yet able to fly but had frequented waters of prairie lakes, where both *Cercaria elvae* and *C. dermolestes* were abundant.

It is remarkable that no females of this species have been found in so large a number of examinations in which males were plentiful. There is the

possibility that they have been overlooked on account of their small size. A more logical explanation, perhaps, is that *Q. discors* is an unnatural host and only the male schistosomes develop in it. This was found to be the case by Faust (53) in infections of human schistosomes in unnatural hosts such as the rabbit and sheep.

Microbilharzia lari and *Ornithobilharzia aviani* are found in local adult Herring Gulls in fairly large numbers. It is not known where the infection is contracted, there being no more evidence to favour the winter feeding ground than the summer habitat. *Diplostomum flexicaudum* is carried in the adult stage by the Herring Gull, and the larvae occur coincidently with schistosome cercariae at Clear Lake. This might indicate that *C. stagnicolae* is the larva of one of the gull schistosomes. Immature male worms of both species were obtained from birds collected in late autumn and, as the time required for maturation in most schistosomes is usually three months or less, this would strengthen the theory that the infection occurred in local waters.

O. filamenta was found in both Herring and Ring-billed Gulls from Clear Lake in midsummer and from Lake Winnipeg in midsummer as well as late autumn. Mature and immature males and females were recovered and the above argument would apply equally to these.

Consistent failure to obtain adult worms by exposure of experimental animals may be due to a number of causes. In addition to the experimental work by the writer, Talbot (150) exposed young domestic ducklings, Herring Gulls, and a pigeon to three species of schistosome cercariae from Douglas Lake, Michigan, but the results were negative in every case. Differences in the temperature, the pH, or the salt concentration of the water used in the laboratory may have been responsible for the production of cercariae so inviable that they were unable to penetrate the skin of experimental animals, or these changes may have interfered with their normal responses to the presence of a host, although this seems unlikely.

Effective treatment of cercarial dermatitis once it has been contracted by human beings is very difficult. The intense itching and annoyance are the most serious aspects, and treatment is usually directed to relieve these. Application of various lotions or ointments is usually recommended, but the writer has found them to be of little or no value in either reducing the irritation or killing the organisms.

The direct pathological effects of schistosomes on the bird host is not known in any detail, but infested birds are invariably lighter and more emaciated than uninfested ones. Much additional work is required on the life-histories and pathogenicity of the local forms.

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THE HELMINTH PARASITES AND PARASITIC DISEASES OF SHEEP IN CANADA

I. A SURVEY AND SOME PRELIMINARY STUDIES ON EXISTING PROBLEMS[1]

By W. E. Swales[2]

Abstract

Investigations have been made upon the identity, incidence, economic importance, and seasonal fluctuations of the helminth parasites in the alimentary canal of sheep in Canada, special consideration having been given to the problems in Quebec and eastern Ontario. Haemonchiasis is the parasitic disease of major importance in Eastern Canada during the summer months, and is replaced by trichostrongylosis, oesophagostomiasis, and possibly hookworm disease in the late autumn and winter. Haemonchiasis and chabertiasis were recorded in British Columbia.

Introduction

Sheep husbandry has not flourished in Canada. At the present time there are no more sheep in the Dominion than there were in 1871, and at that time the important sheep ranges in the Prairie Provinces were not being used. The number of sheep in Ontario in 1937 was 874,769, compared with 1,915,303 in 1882. The decrease in Quebec over the same period was not as great, and the number has approximated 750,000 for the last ten years.

The lack of expansion and actual decrease in sheep production in Eastern Canada have been due to a number of causes, chief among which are probably the difficulty of accommodating and feeding breeding stock during the winter months, a period of low prices obtained for meat and wool, high costs of fencing materials, lack of protection against marauding dogs and predatory animals, increased need of available pastures near cities for dairy cattle, and disease. However, in many outlying areas in Eastern Canada large tracts of land are unsuitable for the production of dairy products but could be adapted for sheep production on a small unit basis, and would produce this "cash crop" with a minimum of expenditure or labour. In those areas the problems of fencing and winter accommodation could be overcome by the present availability of cheap labour and lumber. Shipping costs are now comparatively low, predators are fewer in numbers and prices for good market animals have steadily improved. Canada has been fortunate in having a very low incidence, and in many cases complete absence, of such important bacterial, virus, and protozoal diseases of sheep as anthrax, foot and mouth disease, louping ill, necrobacillosis, variola ovina, heart-water, piroplasmosis, scrapie, and braxy. Psoroptic mange has not been allowed to become established and calliphorine

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myiases are comparatively uncommon. Haemorrhagic septicaemia must be considered as an important problem, although some preventive measures can be applied during periods of exposure. Contagious ecthyma appears to be of moderate importance in range flocks, but is unlikely to become prevalent under eastern conditions.

Diseases due to internal parasites appear to be major causes of losses, particularly in Eastern Canada. Owing to climatic extremes, species that are normally distributed in temperate climates and some that are usually considered as being important only under semi-tropical conditions, are enabled to complete their life cycles on pastures in the Eastern Provinces. It is thought by competent authorities that parasitic disease has been and is still the greatest deterrent to profitable production of sheep in many parts of the Dominion. Extension work has reduced losses considerably in some agricultural districts, particularly in Ontario; the methods of control generally employed have been those in use in the United States of America, and have been directed chiefly against verminous gastritis in lambs due to infestations of *Haemonchus contortus*. However, discouraging losses and lack of thriftiness in lambs and breeding stock have continued throughout many areas, particularly in Eastern Canada.

In the hope that a thorough investigation of the incidence, economic importance, and control of the parasites of sheep in Canada would contribute towards more successful sheep husbandry and would encourage farmers to extend this industry, a series of studies was commenced in 1936. The present paper represents the first of the series, and includes briefly the results of the preliminary work.

The Occurrence of Species

The following species have been found in sheep and lambs in Canada, in natural infections, during the course of these studies.

Parasite	*Estimated Prevalence*
CLASS—TREMATODA	
Dicrocoelium dendriticum	Recorded in Nova Scotia only.
Fasciola hepatica	Confined to small areas.
CLASS—CESTODA	
Moniezia expansa	Very common.
Thysanosoma actinioides	Common in Western Canada.
Cysticercus tenuicollis	Very common.
C. ovis	Rare.
CLASS—NEMATODA	
Ascaris sp.	Rare.
Strongyloides papillosus	Very common.
Oesophagostomum columbianum	Very common in Eastern Canada.
O. venulosum	Common in British Columbia.
Chabertia ovina	Very common.
Monodontus trigonocephalus	Very common.
Dictyocaulus filaria	Not common.
Muellerius capillaris	Very common.
Trichostrongylus axei	Very common.

Parasite	*Estimated Prevalence*
CLASS—NEMATODA—*conc.*	
T. vitrinus	Very common.
T. colubriformis	Very common.
Cooperia curticei	Very common.
C. oncophora	Not common.
Ostertagia circumcincta	Very common.
Haemonchus contortus	Very common.
Nematodirus filicollis	Very common.
N. spathiger	Common.
Gongylonema pulchrum	Rare.
Trichuris ovis	Very common.
Capillaria longipes	Not common.

The above list includes only two additions to other published records in Canada (Swales (12) and Fallis (5)), these being *Gongylonema pulchrum* and *Capillaria longipes*. It is, therefore, considered to be an accurate list of all species that are liable to be encountered in reasonably large samples. The expected discovery of other species of *Trichostrongylus* and *Ostertagia* has not been made.

Special Technique Employed in Autopsies

In estimating the numbers of individuals of the larger species, *Haemonchus contortus*, *Moniezia* spp., *Monodontus trigonocephalus*, *Trichuris ovis*, *Chabertia ovina*, and *Oesophagostomum columbianum*, direct counts of all individuals screened from intestinal washings were made. In light infections *Ostertagia circumcincta* and *Nematodirus* spp. were also counted individually. In order to estimate the numbers of the small species, *Trichostrongylus* spp., *Cooperia* spp., and *Strongyloides papillosus*, it was necessary to employ a dilution technique.

Owing to the importance of making accurate estimations the examination of a small portion of a formalin suspension of the intestinal contents was not found adequate. After numerous tests a simple technique was evolved which has proved to be a reliable and rapid method, and which has been adopted as the standard technique in these studies. This method is described in some detail owing to the interest expressed in it by several visiting parasitologists.

After removal from the animal the abomasum is ligated at the omaso-abomasal orifice and at the pylorus, the duodenum being left attached to the abomasum. The ileum is ligated at or near the ileo-caecal valve. The whole is then immersed in 5 to 10% formalin in a tank large enough to conform to the rules necessary for adequate fixation; specimens are left in this solution for at least two days.

The preserved abomasum is examined by opening it over a pail, and a stream of water is run on to the mucous membrane as it is thoroughly rubbed with the fingers. The suspension of ingesta and parasites is then screened through a stack of sieves (Fisher's U.S. Standard Sieve Series Nos. 10, 20, and 40) which have mesh diameters or nominal openings of 2.00 mm., 0.84 mm., and 0.42 mm. respectively, and with wire diameters of 0.76 mm., 0.42 mm., and

0.25 mm., and which are 21.7 cm. in over-all diameter. The type of sieve as devised by Hall and Cram is not suitable for this method.

As the sieves are filled, a well-fitting cover is placed over them and they are shaken with a rotary motion. Water is added until most of the soluble material is removed. Each sieve is now inverted in a large moist chamber dish made of thick glass and the contents are washed off with water which has been cleared of air bubbles. The contents of each dish are allowed to settle and the supernatant water is then decanted. The large species are now counted, the dish being placed over a black surface and under a strong light, for this purpose. The contents of the dish are then transferred to another dish which has a diameter of 25 cm. and a bottom area of 491.0 sq. cm. The contents, which should be approximately half an inch in depth, are gently shaken until they are evenly distributed in the dish; a metal ring is quickly placed in the mass in such a way that its edge is near the centre of the dish. This ring, which has an area of 49.1 sq. cm. thus separates one-tenth of the contents of the dish, and this amount is pipetted into a square Petri dish which has been ruled to fit the field of a dissecting microscope. The ring used in this work is the screw-top of a one-pint glass preserving jar, the bottom edge having been levelled and sharpened.

The one-tenth sample from each dish is then examined systematically under a magnification of ×25 for the detection and identification of the genera of small parasites.

In 20 tests with 1,000 adult *Osteriagia circumcincta*, this sampling technique indicated the number within 10% in 19 tests, the mean error being ±4.45%.

This technique requires some practice and experience in spreading the ingesta in the dish and placing the ring, in order to obtain accurate results, but its use approximately doubled the number of specimens that it was previously possible to record by the use of other reasonably accurate methods.

Observations on the Abundance and Pathogenicity of Parasites in Lambs in Eastern Canada

In 1936 a preliminary survey of the parasites in 134 abomasa of market lambs was made. The lambs came from farms in the southern sections of Quebec and in eastern Ontario. The collections were made at random in abattoirs in Montreal. The results of the examinations of the contents, made under laboratory conditions, are shown in Table I.

The low numbers of individuals recovered from these animals would be due, in part at least, to the fact that the lambs were relatively healthy specimens and were slaughtered between October 23 and November 25.

As noted by Fallis (5) and as illustrated later in this paper, the peak of abundance of *H. contortus* occurs in the summer and then the number of individuals decreases rapidly in the months of autumn. *O. circumcincta* has a similar fluctuation, but *T. axei* occurs in greatest numbers during the late autumn or winter months.

TABLE I

ABOMASAL PARASITES OF 134 LAMBS FROM QUEBEC AND EASTERN ONTARIO

Parasite	No. of lambs infected	Percentage infected	Mean no. of worms per infected lamb	Min. and max. nos. of worms
Haemonchus contortus	82	61.2	45.4	1 – 530
Ostertagia circumcincta	99	73.9	154.2	3 – 1,620
Trichostrongylus axei	124	92.5	605.4	20 – 8,710

The results of these preliminary observations on abomasal parasites agree in all important details with those of Fallis in Ontario, seasonal variations being considered, and for this reason no further details are included.

The degree of infection with certain species of nematodes which is able to cause clinical disease or at least mark unthriftiness in lambs or adult sheep, has been the subject of some study. An examination of the literature reveals that some workers have set a tentative line between tolerated and harmful infections for known pathogenic species. It is usually considered that mixed infections are more harmful than infections with a single species, and this supposition, supported by some evidence of lowered resistance to one species being produced by another, will remain for the present a complication in preliminary observations on pathogenicity. Ross and Gordon (10) state that it is highly improbable that infections with less than 500 *H. contortus* are ever the primary cause of serious effects, and that fatal cases are seldom seen with less than 1,000 individuals of this species. They point out the importance of the complicating factor of nutrition in such estimation.

Robertson (9) estimates that fatal results are not produced by less than 8,000 individuals of *O. circumcincta*. Taylor's (14) estimations of numbers of this species would indicate that fatal results are produced by this species, but that about 16,000 individuals are necessary.

Trichostrongylus spp. are the undoubted cause of severe gastro-enteritis in Great Britain, Africa, and Australia, and under suitable climatic conditions must always be considered as potential pathogens. Ross and Gordon state that fatal infections are seldom induced in Australia by less than 10,000 of these worms in the abomasum and small intestine, and state at the same time that Taylor's work indicates that in Great Britain the number is considerably larger.

Experimental evidence that *Cooperia curticei* in very heavy infections (10,000 to 25,000 individuals) affects the economy of lamb production has been brought forward by some workers, notably Andrews (1).

There is a lack of experimental evidence of the effect of the parasites of the large intestine, but it is generally agreed that heavy infections with *Chabertia ovina* or *Oesophagostomum columbianum* are very harmful. Ross considers that more than 100 individuals of either of these parasites constitute a heavy infection.

For the purpose of comparisons for the preliminary work, the above findings and opinions were used for the adoption of tentative standards for conditions in Canada. Thus, infections which were considered harmful are:—

Haemonchus contortus	over 500 individuals
Ostertagia circumcincta	over 8,000 individuals
Trichostrongylus spp.	over 10,000 individuals
Cooperia curticei	over 10,000 individuals
Chabertia ovina	over 100 individuals
Oesophagostomum columbianum	over 100 individuals

In comparison with the above mentioned nematodes, all of which are commonly encountered in Canada, the other common parasites, *Moniezia expansa*, *Thysanosoma actinioides*, *Nematodirus* spp., *Strongyloides papillosus*, *Trichuris ovis* and *Muellerius capillaris*, were not considered as of primary importance but were always considered in subsequent observations. *Monodontus trigonocephalus* could not be placed in either of these groups owing to lack of data on its pathogenicity.

A large number of very poor lambs from the Eastern Provinces are placed on the market each year. From time to time viscera from such animals were obtained from abattoirs in order to collect specimens for identification, and in most cases a large number of nematodes or lesions due to the invasion of *Oesophagostomum columbianum* were present. It was thought that most of these animals were suffering from the effects of severe helminthiasis, although it was realized that observations made upon such animals would be of limited value without knowledge regarding other conditions, particularly those of nutrition, to which they had been subjected. However, in order to collect data on the limits of infection in these and in healthy stock from the same districts a number of observations were made. Lambs only were studied, and these were divided into "Cull lambs" which were thin and under 30 lb. dressed weight, and "Market lambs," which were healthy in appearance and over 30 lb. dressed weight. In the unhealthy lambs the dressed weight was, in most cases, somewhat less than 45% of the live weight, and in the healthy lambs it was usually slightly more than 50%.

The results of the examinations of these lambs are presented in Tables II, III, and IV. Each table represents animals killed during the same month, so that seasonal fluctuation of species need not be considered in the comparisons within each table.

It will be noted that the significant difference between the "Cull" and "Market" lambs in Table II (August) is in the number of *Haemonchus contortus* only. In Tables III and IV (September and October) this significant difference is not present, nor is there any other significant difference. This may be explained by the seasonal fluctuation of *H. contortus*, and would indicate that if any one species of parasite was responsible for the condition of the "Culls" it was *H. contortus*, the pathological effects having been produced earlier in the season.

TABLE II

LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE. Killed in August, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Mono-dontus*	*Nemato-dirus* spp.	*Cooperia* spp.	*Tricho-strongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oeso-phago-stomum*	Nodules
"Cull"													
1	103	60	230	0	5	220	260	430	0	40	0	1	22
2	285	9,550	900	7	8	146	310	230	1,060	4	0	20	53
3	90	230	260	0	5	108	280	300	40	53	1	0	6
4	1,295	1,020	540	1	37	432	160	1,060	80	31	0	11	175
5	177	67	90	4	11	138	290	80	70	104	0	0	54
6	4,134	4,880	520	5	105	317	3,790	1,050	40	19	26	0	71
7	185	147	150	0	2	590	0	180	760	27	0	0	5
8	2,284	920	60	7	10	1,313	50	40	20	8	0	0	14
9	143	10,970	450	7	36	50	320	600	160	2	0	28	316
10	3,547	165	100	0	18	83	140	0	480	10	1	0	14
Totals	12,243	28,009	3,300	31	237	3,397	5,600	3,970	2,710	298	28	60	730
Means	1224.3	2800.9	330	3.1	23.7	339.7	560	397	271	29.8	2.8	6	73
"Market"													
1	19	940	360	0	73	22	90	50	140	89	0	7	138
2	3	780	540	3	16	15	320	120	60	9	0	7	184
3	241	330	80	8	12	26	0	2,770	0	2	5	0	117
4	30	303	80	0	4	258	30	280	40	2	6	0	8
5	188	621	0	0	47	36	0	80	30	0	0	0	1
6	21	334	80	0	3	66	40	30	20	22	0	0	6
7	57	1,172	630	14	23	324	10	260	70	27	7	0	13
8	23	60	160	0	6	116	50	250	400	2	1	0	11
9	20	566	0	0	7	59	40	140	0	7	2	0	0
10	651	1,470	460	90	4	1,210	270	900	0	17	11	1	32
11	280	410	100	16	21	349	0	250	50	16	1	0	6
12	3	10	10	0	0	0	0	0	0	1	0	1	40
13	21	30	10	2	2	19	0	0	0	5	1	0	23
14	494	2,020	0	0	0	1,280	300	320	0	4	0	0	0

TABLE II—*Concluded*

LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE. Killed in August, 1938—*Concluded*

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"*Market*"—con.													
15	462	500	30	0	0	60	20	50	0	6	0	0	7
16	469	230	90	0	0	30	0	100	0	12	1	1	10
17	269	261	160	2	2	23	10	30	20	9	0	1	34
18	157	237	0	0	0	1,070	0	0	0	39	0	0	2
19	10	123	120	0	0	8	0	0	0	9	0	2	40
20	138	3,010	4,360	16	0	30	120	2,180	0	5	0	8	147
21	76	34	0	3	0	15	0	40	30	2	0	0	37
22	980	340	30	0	0	32	20	10	20	2	0	0	13
23	45	780	350	0	13	32	30	90	250	8	0	0	14
Totals	4,657	14,561	7,650	154	233	5,080	1,350	7,950	1,130	295	35	28	892
Means	202.5	633.1	332.6	6.7	10.1	220.9	58.7	345.6	49.1	12.8	1.5	1.2	38.8

SAMPLE TESTS FOR SIGNIFICANT DIFFERENCES

Lambs	Parasite	Means	S.D.	Mean diff. ± S.E. diff.	P
"Cull"	*Haemonchus*	1224.3	1555.1	1020.9 ± 498.6	0.05
"Market"	*Haemonchus*	203.4	397.2		
"Cull"	*Ostertagia*	2800.9	4203.2	2167.5 ± 1331.0	0.1
"Market"	*Ostertagia*	633.4	354.6		

TABLE III

LAMBS FROM BEAUCE COUNTY AND MANIWAKI

Killed in September, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"Cull"													
1	188	1,100	0	0	63	34	20	50	450	6	25	1	4
2	21	2,200	460	12	11	320	160	4,950	0	51	14	0	61
3	428	100	60	5	35	20	200	120	20	1	16	6	116
4	2,014	940	290	18	134	42	1,150	3,180	450	3	3	32	34
5	1,276	1,350	940	35	52	24	90	1,590	80	5	5	6	454
Totals	3,927	5,690	1,750	70	295	439	1,620	9,890	1,000	66	63	45	669
Means	785.4	1,138	350	14	59	88	324	1,998	200	13.2	12.6	9	133.8
"Market"													
1	0	0	170	0	6	10	0	190	0	2	2	3	275
2	140	1,020	50	89	54	571	190	730	230	4	16	66	195
3	167	1,100	620	16	21	31	90	600	160	28	7	54	321
4	1,349	1,390	170	0	8	5	510	230	0	33	3	4	196
5	26	1,500	300	9	25	169	310	420	310	30	9	29	309
6	82	70	370	0	60	40	370	560	80	0	21	5	177
Totals	1,764	5,080	1,680	114	174	826	1,470	2,730	780	97	58	161	1,473
Means	294	846.6	280	19	29	137.7	245	455	130	16.1	9.7	26.8	245.5

TABLE IV

LAMBS FROM FRONTENAC COUNTY, QUE. (FOUR FLOCKS, CONTROLS FOR EXPERIMENTAL TREATMENTS)

Killed in October, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"*Cull*"													
1	1,414	2,650	4,190	7	53	75	10,950	4,060	280	10	5	25	1,613
2	130	1,500	2,630	12	32	60	2,720	3,790	220	1	0	2	1,299
3	138	610	1,820	5	99	430	4,330	810	230	1	1	3	641
4	240	890	1,440	10	8	30	3,600	2,380	60	11	0	2	1,561
5	226	1,920	3,190	10	43	60	3,310	720	200	11	0	24	1,630
6	130	950	3,990	6	28	210	1,780	350	80	9	0	5	748
7	186	660	1,280	5	57	30	1,310	530	220	1	0	5	726
Totals	2,464	9,180	18,540	55	320	895	28,000	12,640	1,290	44	6	66	8,218
Means	352	1311.3	2648.6	7.9	45.7	126.4	4,000	1805.7	184.3	6.3	0.9	9.4	1,174
"*Market*"													
1	140	730	2,600	6	16	20	1,030	2,290	60	4	0	4	1,431
2	109	270	1,340	12	33	310	3,340	1,160	240	13	0	6	1,040
3	152	560	1,870	12	32	10	1,840	1,250	110	9	1	2	1,379
4	792	1,370	4,380	9	175	0	850	1,720	440	11	2	19	930
5	105	510	3,340	0	33	38	900	2,710	50	33	0	5	1,098
6	68	270	5,970	19	17	30	2,140	1,760	680	12	1	2	1,003
7	40	80	4,190	0	5	0	210	1,440	0	11	1	1	169
8	110	250	3,180	3	25	20	80	440	0	13	1	0	50
9	727	260	430	10	5	0	50	560	70	44	0	0	154
Totals	2,243	4,300	27,300	71	341	428	10,440	13,330	1,650	150	6	39	7,254
Means	249.2	477.7	3033.3	8	38	47.6	1,160	1481.1	183.3	16.6	0.66	4.3	806

In order to support the above hypothesis, and for purposes of comparison with data obtained by Fallis (5) and other authors on seasonal fluctuation, further observations were made upon the incidence of the various species in a single flock. All the lambs from this flock were slaughtered as good market lambs, and the observations were made over a period of two seasons and thus on two crops of lambs from the same stock and on the same pasture. The data are presented graphically, each graph showing the observed fluctuations in the mean number of individuals of a species from July to March (Figs. 1–11). The number of animals observed was 275, being distributed as follows:—

July	27	October	54	January	14
August	33	November	24	February	15
September	93	December	8	March	7

Although the observations in some months were, of necessity, small, these graphs serve to illustrate the tendencies of fluctuations more accurately than data from mixed lots of lambs from widely separated districts. Such variations as the fall in the number of *Nematodirus* spp. in August, for example, cannot be accepted as true deviations from a curve, but the gross contours of the curves appear to illustrate the usual tendencies in eastern Ontario and Quebec, and agree closely with those compiled by Fallis in southern and western Ontario.

Notes on Parasitic Diseases of Lambs in Late Autumn and Winter in Eastern Canada

From the above data one would not expect to find verminous gastritis due to *H. contortus* or *O. circumcincta* in lambs in Eastern Canada during the late autumn or winter months. Nevertheless a large number of animals, from 7 to 11 months of age, are marketed each year during the cold months in extremely poor condition. Some are so emaciated that they weigh less than 20 lb. dressed, and are frequently held under observation by Federal Inspectors for mucoid degeneration. Table V shows the results of necropsies on sample specimens of these emaciated lambs, which were all less than 20 lb. dressed weight. These findings indicate two features of particular interest, the superinfection of *Trichostrongylus* spp. in three animals, the heavy infection of *Monodontus* in three, and the very large number of lesions due to *Oesophagostomum columbianum* in all the animals. These lesions, when present in large numbers, literally cover the intestines of small animals, frequently extending from the abomasum to the rectum. In very heavy infections there is little doubt regarding the pathogenic effect of the causative agent, particularly in those numerous cases in which partial or complete occlusion of the ileo-caecal valve has been produced. The number of lesions on the small intestines is approximately the same as on the large intestines, but the region around the ileo-caecal valve appears to be constantly filled with nodules.

Owing to the fact that the effects of infection by *Oesophagostomum columbianum* in lambs in Eastern Canada appear to differ in several important details from those in other parts of the world, this disease has been the subject

TABLE V

EMACIATED LAMBS FROM EASTERN CANADA SLAUGHTERED IN DECEMBER AND FEBRUARY

Month of slaughter	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodular lesions
December	0	0	3,310	42	49	0	490	12,140	0	10	27	17	1,008
	60	190	60	4	214	0	150	740	0	7	0	20	905
	0	10	1,910	0	324	70	9,140	10,150	0	776	36	4	541
	0	60	2,340	10	23	200	1,130	3,530	50	29	6	3	704
	0	50	2,450	1	27	0	1,820	8,830	?	9	12	13	931
	10	210	1,020	2	28	0	1,820	4,030	150	4	16	7	1,119
February	0	40	4,000	1	197	210	2,550	2,010	?	0	3	104	306
	19	760	2,800	0	.22	0	0	5,700	?	4	0	468	1,682

of special studies, the results of which will be reported in a later paper. As the position of the lesions on the intestinal tract appears to exert a greater influence on the health of the host than mere numbers, a tentative line between tolerated and harmful infections is difficult to set. It is apparent, however, that less than 300 lesions do not affect the normal development of the young animal, and that under certain conditions a well grown lamb can tolerate a much larger number.

The Parasites of Adult Stock in Eastern Canada

Because of the methods of marketing sheep in Canada, whereby the young stock is sold as "lamb" from July until the following spring at from 4 to 11 months of age, it is difficult to obtain viscera from relatively young breeding stock for parasitological studies. These marketing practices are due to economic necessity, because the cost and labour of overwintering any sheep other than those to be used for breeding could not be recovered from future sales. Thus a suitable market for "mutton" has not been developed, and adult sheep are usually marketed only when they have become unprofitable to their owners and must be sacrificed at a low price. Sheep which appear on the markets in the spring, early summer and in the winter are frequently in poor condition, and the majority are more than three years old.

Adult sheep in Eastern Canada are almost invariably infected with lesions due to *O. columbianum*; during the course of these studies only two lots of sheep from the Province of Quebec that included individuals free from this infection have been seen. These lots came from Lac St. Jean and Amqui, two more northerly points of agricultural Quebec; all other lots have shown numerous lesions in all individuals.

During the course of field investigations, small numbers of ewes have been killed and examined on farms in attempts to determine the cause of emaciation and inability to nourish their lambs. Severe infection with lesions of oesophagostomiasis have been the only constant pathological process noted. Samples of emaciated ewes placed on the market in Montreal during the spring and early summer have been examined. The number is too small for any definite decision regarding the cause of the emaciation, but, in the absence of any obvious pulmonary infection, the findings in regard to helminthic infections are of some interest. The results of necropsies on eight ewes over four years of age are shown in Table VI. These findings appear to indicate the probable effect of the numerous lesions of oesophagostomiasis in the presence of only light infections of other helminth parasites in the intestinal tracts.

The Parasites of Sheep and Lambs from Western Canada

Large numbers of lambs from the ranges in southern Saskatchewan and Alberta are slaughtered in Eastern Canadian abattoirs each year. These animals are usually excellent fat lambs of the Rambouillet breed. The most striking feature noted during the abattoir operations is that these animals are entirely free from lesions of oesophagostomiasis. There is, however, a

TABLE VI

EMACIATED ADULT EWES FROM EASTERN CANADA

—	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodular lesions
1	0	4	97	0	13	0	12	8	?	0	0	3	1,180
2	0	16	50	0	8	10	40	110	?	0	0	10	1,088
3	2	10	70	0	8	0	0	20	?	0	0	0	520
4	0	10	310	0	5	10	0	30	?	0	0	0	1,685
5	0	7,900	4,720	0	45	50	0	210	?	0	1	6	887
6	0	1,750	2,850	0	52	150	70	300	?	0	69	48	760
7	194	460	950	0	12	0	170	10	?	0	0	17	543
8	10	150	0	0	23	0	340	0	40	0	0	0	1,099

fairly high incidence of infection in the intestine and bile ducts with the Fringed Tapeworm, *Thysanosoma actinioides*. A sample of 18 animals from a "feed-lot" in western Ontario, which had been received previously from southern Saskatchewan, was examined in 1934. All these animals were infected with *T. actinioides*; although tapeworms were present only in the bile ducts of five livers, the majority were in the small intestines. Very light infections of both *H. contortus* and *O. circumcincta* were present in all the abomasa, but the greatest number of these stomach worms in a single lamb was 30. Fourteen of the small intestines contained small numbers of *Nematodirus filicollis*, 12 contained small numbers of *Trichostrongylus colubriformis*, and six contained up to ten *Monodontus trigonocephalus*. All the large intestines contained small numbers of *Trichuris ovis* and two contained up to five *Chabertia ovina*. In addition to the above, one specimen of *Moniezia expansa* and two of *Capillaria longipes* were recovered from the lot.

The only infection of interest in these fat lambs was that of *T. actinioides*; 100 of these parasites were present in one small intestine and two were in the liver of the same animal.

During these studies the abattoir operations on several hundreds of fat western lambs have also been observed and a high incidence of *T. actinioides* in the bile ducts has been noted. These observations have led the writer to agree with Christiansen (3) that this parasite is not of major importance.

Viscera from western sheep and lambs that were reputed to be suffering from parasitic disease have been examined from time to time. The results of these examinations are shown in Table VII, the numbers of parasites in all but the last four animals being approximate.

The animals from Saskatchewan and Alberta were examined for the purpose of routine diagnosis and the numbers of individual parasites were not accurately recorded. However, in no case was an infection of pathological significance noted. The cases from British Columbia are very interesting. The animal from Agassiz was pastured on land which is subject to a very high summer rainfall and relatively high summer temperatures, and was undoubtedly suffering from haemonchiasis. In addition, the number of hookworms present was of probable clinical significance.

The three lambs from Vavenby had been subjected to entirely different conditions, and had ranged over land in the drier interior of the province. They were samples of a number of animals that had suffered from an enteritis characterized by scouring, with dark fetid faeces. The diagnosis in these three cases was severe chabertiasis, extensive damage having been produced in the crown of the small colon. These cases are of particular interest because they constitute definite evidence of the occurrence of a parasitic disease in a part of the Dominion that is of major importance in sheep husbandry. The number of individuals of *Chabertia ovina* present in each animal appears to be greater than any cases recorded from other countries.

TABLE VII

PARASITES IN THE INTESTINAL TRACTS OF UNHEALTHY SHEEP AND LAMBS IN WESTERN CANADA

Animal	Origin	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Thysanosoma*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*
Lamb	Edmonton, Alta.	0	50	10	0	2	0	1,539	40	0	0	3	0
Lamb	Edmonton, Alta.	0	410	0	0	0	0	1,149	30	150	0	0	0
Lamb	Tilly, Alta.	50	400	?	1	0	0	Few	Few	Few	?	5	0
Lamb	Tilly, Alta.	200	2,000	?	Few	0	0	700	12	?	?	12	7
Lamb	Cypress Hills	150	75	?	0	0	0	30	?	?	?	0	0
Lamb	Hatton, Sask.	0	Few	?	3	0	0	130	?	Few	?	0	0
Lamb	Piapot, Sask.	10	Few	?	10	0	0	40	?	?	?	1	0
Ewe	Maple Creek, Sask.	40	14	?	1	0	0	40	?	?	?	0	0
Ewe	Agassiz, B.C.	1,487	4,880	520	5	0	105	317	3,790	1,050	40	19	26
Lamb	Vavenby, B.C.	0	1,750	830	0	0	0	?	?	?	?	30	610
Lamb	Vavenby, B.C.	0	7,950	2,390	4	0	0	2,080	800	600	?	7	682
Lamb	Vavenby, B.C.	0	3,420	3,510	3	6	0	2,500	3,010	3,190	?	75	658

Discussion

In these preliminary studies an attempt has been made to show which species of parasitic worms are commonly present in sheep and which are of major importance. Data have also been presented in order to explain causes of unthriftiness in groups of animals, chiefly from Eastern Canada. From these data it is apparent that verminous gastritis, due to infection with *Haemonchus contortus*, is an important disease of lambs during the grazing season in the Province of Quebec and probably in all parts of Eastern Canada.

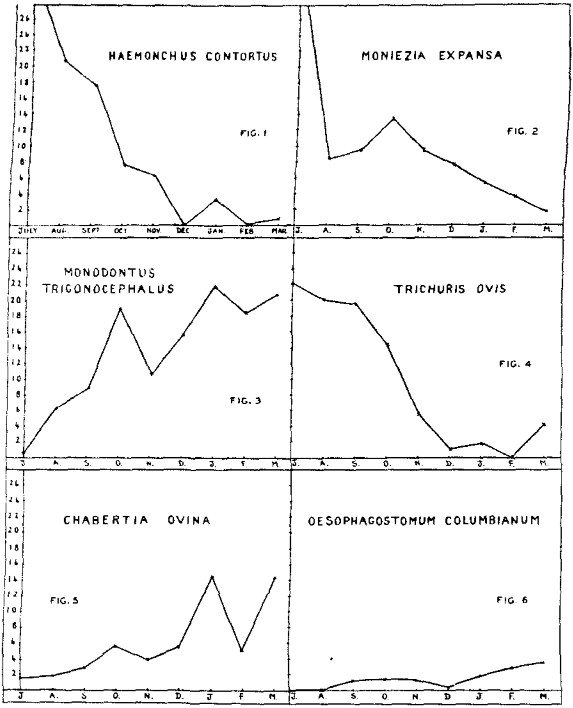
Infection with *Ostertagia circumcincta* has not been found of major importance, but, having reference to findings in Great Britain and elsewhere, this paper has not shown that this parasite cannot become of importance under certain conditions during the summer.

In the autumn and winter months in Eastern Canada verminous gastritis becomes of lesser importance, but heavy infections of *Trichostrongylus* spp. and the lesions due to invasion of the intestinal walls by larvae of *Oesophagostomum columbianum* become the apparent causes of unthriftiness.

In adult sheep in Eastern Canada the lesions of oesophagostomiasis appear to be a cause of marked unthriftiness during the breeding season.

The nature of parasitic disease in sheep in the Prairie Provinces has not been made apparent. In British Columbia records of importance have been made; in one region which is subject to a high summer rainfall, haemonchiasis and possible hookworm disease were recorded, and in a drier region in the interior of the province diagnoses of severe chabertiasis were made.

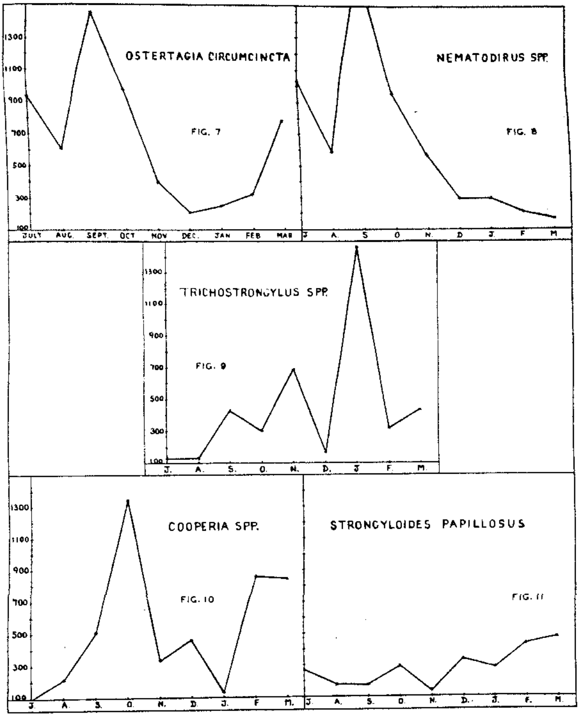
These preliminary surveys were made necessary by the fact that certain species of parasites that are of known pathogenic importance in one region may be present but of little or no importance in another region which is subjected to a different climate and different methods of husbandry. The pastures in Eastern Canada are not used during the winter months, when they remain under snow for a long period. Griffiths (6) has shown that *Moniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis* and *Trichostrongylus colubriformis* are able to survive a normal winter on a pasture in the Province of Quebec. If the free-living stages of a certain species do survive and are present on the pastures in the spring, then one might expect this species to be present in reasonable numbers in lambs after they have grazed on the pasture for from six to eight weeks. Figs. 1 to 11 show the relative incidence of the common species in July, and would indicate, according to the above hypothesis, that *M. expansa*, *T. ovis*, *O. circumcincta*, *Nematodirus* spp. and *H. contortus* survived the winter. Accordingly, then, it is also apparent that winter survival in the case of *M. trigonocephalus*, *C. ovina*, *O. columbianum*, *Trichostrongylus* spp. and *Cooperia* spp. is low or non-existent. It will be noted, however, that *H. contortus* was present only in abnormally small numbers in these animals, and is numerically comparable to five other species that are usually present in small numbers (compare with data obtained from



FIGS. 1–6. *The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.*

market animals). Owing to this abnormality, which is probably due to the fact that the flock was treated with anthelmintics known to have a positive action upon *H. contortus*, this species cannot be considered in the hypothesis regarding winter survival.

The factor of non-survival on the pastures, combined with factors of summer rainfall and temperature, would have a profound effect upon the ability of certain species to be of pathogenic importance. These preliminary studies have, then, demonstrated the importance of knowledge regarding the biology



FIGS. 7–11. *The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.*

of the free-living stages of each species of helminth parasite present in sheep in Canada.

It is already apparent that owners of sheep in Eastern Canada must adopt efficient means of combating haemonchiasis in their animals during the summer months, and that they have urgent need of some measures of control of oesophagostomiasis in order to protect the health of breeding stock. The possible occurrence of disease due to heavy infections of *Trichostrongylus* spp., *M. trigonocephalus*, and *C. ovina* must always be considered.

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THE INTERRELATION OF LIPIDS IN THE BLOOD PLASMA OF WHITE LEGHORN COCKERELS[1]

By Eldon M. Boyd[2] and Eleanor L. Clarke[3]

Abstract

The blood plasma of 22 healthy, young, white leghorn cockerels was analysed by oxidative micromethods and found to contain the following mean lipid values, in milligrams per 100 ml. of plasma: total lipid 520, neutral fat 225, total fatty acids 361, total cholesterol 100, ester cholesterol 66, free cholesterol 34, phospholipid 155. In general, the amounts of the various lipids were proportionately related to the total lipid.

In recent communications, Boyd has shown that when values for the various lipids of blood plasma of man (3) and rabbits (4) are plotted against increasing values for total lipid, it is demonstrated that in normal animals a *proportionate increase occurs in the value of each of the component* lipids. During certain experiments recently performed in this laboratory, a group of some two dozen healthy, normal, white leghorn cockerels became available, and it appeared desirable to make a further study of the interrelation of plasma lipids in these birds belonging, as they do, to a class of vertebrates different from that usually experimented upon in studies of lipid metabolism. A differential lipid analysis of the blood plasma of cockerels has not been made with oxidative micromethods. The few studies of blood lipids in birds that have been done have been concerned chiefly with the effect of egg-laying (e.g., 9, 11) and in none of these has a complete analysis been performed.

It was also felt that an analysis of the plasma lipids of birds would provide information of value in connection with another aspect of lipid metabolism, namely, the relation of plasma lipids to diet. The lipid content of plasma is in general somewhat, and often markedly, lower in herbivorous animals such as rabbits (4), guinea pigs (6), goats (1), cows (8), and steers (10) than in omnivorous animals such as man (3) and dogs (7). Bloor (2) has found that the blood lipid levels of rabbits may be altered by changes in diet to a greater extent than those of dogs.

Methods

The cockerels used were obtained from a poultry farm in the spring of the year, when they were one pound in weight. They were housed in a large

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room, with complete liberty of movement, and were fed on Purina Chick Growing Mash and whole wheat grain with crushed oyster shell and water ad libitum. They were killed in the early autumn, and weighed between 1200 and 1600 gm. each. At this time they appeared to be in excellent condition, crowed lustily, and had well developed combs. An autopsy was performed immediately after drawing blood for lipid analysis, and there were no pathological findings. Potassium oxalate was used as an anticoagulant for the blood, which was collected from the neck vessels. The blood was immediately centrifuged and the plasma extracted and analysed by oxidative micromethods (5).

It is an accepted and unfortunate fact that "blood lipid" values vary according to the technique employed, especially colorimetric estimations of cholesterol versus the oxidative method. A discussion of some factors that may affect lipid estimations has recently been made (5). Not only is there a variation from technique to technique, but occasionally two or more analysts may fail to obtain the same results with the same technique. After sufficient experience however, a qualified analyst may repeat his own results within a coefficient of variation of less than 5%. Comparative studies of blood lipids by the same analyst with the same technique yield data which do not require the necessity of estimating the effect of differences in technique.

Results

A statistical summary of the results is given in Table I. Most of the values were notably similar to those in normal human blood, analysed by these same methods, and as notably different from the lipid values of rabbit and guinea

TABLE I

THE LIPID COMPOSITION OF BLOOD PLASMA IN 22 YOUNG WHITE LEGHORN COCKERELS

(Results are expressed in mg. per 100 ml.)

Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospholipid
				Total	Ester	Free	
Minimum	368	115	248	65	31	19	90
Maximum	704	374	503	154	106	54	240
Mean	520	225	361	100	66	34	155
Standard deviation	85	77	74	23	19	9	34
Coefficient of variation	16	34	20	23	29	26	22

pig blood. The various cholesterol fractions and phospholipid were somewhat lower in value than those in man and the neutral fat somewhat higher. All of the values were considerably higher than previously found by the author in the blood plasma of rabbits and guinea pigs (4, 6). We have to the present determined lipid values in the blood plasma of four different species, and the

various means of these analyses have been collected in Table II. It is hoped that an opportunity may later be provided to extend this comparative study.

TABLE II

A COMPARISON OF THE MEAN VALUES FOR PLASMA LIPIDS IN HUMAN, COCKEREL, RABBIT, AND GUINEA PIG BLOOD BY THE AUTHORS' OXIDATIVE MICROMETHOD

(Results are expressed in mg. per 100 ml.)

Species	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
Human (3)	530	142	316	152	106	46	165
Cockerel	520	225	361	100	66	34	155
Rabbit (4)	243	105	169	45	23	22	78
Guinea pig (6)	169	73	116	32	21	11	51

In order to show the relation of values of the different lipid fractions one to the other, the results for cockerel plasma were divided into groups of increasing lipid values. Moving averages within these groups were then calculated by averaging the values in groups with total lipid values between

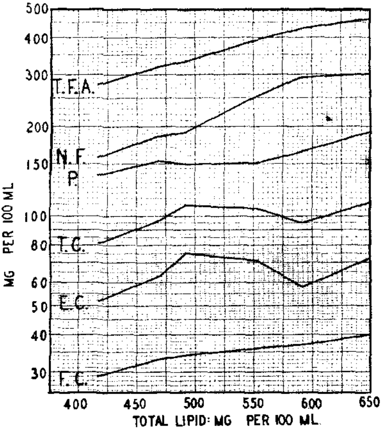

FIG. 1. *Arithlog plotting of changes in the moving averages of values for lipids in the blood plasma of healthy young cockerels with changes in the moving averages for total lipid.* T.F.A.—*total fatty acids;* N.F.—*neutral fat;* P.—*phospholipid;* T.C.—*total cholesterol;* E.C.—*ester cholesterol;* F.C.—*free cholesterol.*

350 and 450 mg. per 100 ml., between 400 and 500 mg., between 450 and 550 mg., and so on. The resulting moving means were then plotted on arithlog paper and this chart is given in Fig. 1. It may be seen again that in general when there is more of one lipid present in plasma there is correspondingly more of another. As in rabbit plasma (4), the chief fluctuations occurred in the values for ester cholesterol, which erratically increased with increase in total lipid. It should be emphasized again that these interrelations have been demonstrated in *normal* animals. In conditions accompanied by a lipopenia or lipemia, such correlation may not—and in instances where it has been studied does not (3)—exist.

The results indicate further that plasma lipids in normal herbivorous animals need not necessarily be lower than those in omnivorous animals.

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THE CARBOHYDRATE METABOLISM OF DEVELOPING SALMON EGGS[1]

By F. R. Hayes[2] and Andrew Hollett[3]

Abstract

The glycogen and glucose contents of salmon eggs, detached embryos, yolk sacs, and livers have been estimated periodically from fertilization to the end of yolk absorption. Glycogen is absent at the beginning, appears midway between fertilization and hatching, and later increases steadily in concentration, reaching a maximum of 0.53 mg. per 130 mg. egg. Glycogen storage by the liver begins suddenly some time after hatching, before which the muscles serve as a storage place. No glycogen ever appears in the yolk. The initial quantity of glucose is 0.064 mg. per egg. The concentration increases with development, is interrupted at hatching, and then continues to rise, reaching a maximum value of 0.29 mg. per egg. The glucose is distributed throughout all parts of the system, and its fluctuations parallel those of the water content, leading to the suggestion that the glucose concentration per unit of water is constant throughout development.

This investigation was carried out to determine the carbohydrate content of salmon eggs and larvae up to the end of the yolk-sac stage, in order to obtain information regarding the metabolism of carbohydrate and its place of storage. The free sugar content and the glycogen content were determined of: (i) the whole egg or larva (embryo plus yolk); (ii) the embryo only; (iii) the yolk only; (iv) the liver. Where the word "embryo" is used in this paper, it means "embryo dissected off the yolk sac".

Carbohydrate in eggs and animal tissues may be present in three forms, namely free glucose, free glycogen, and a carbohydrate complex forming part of a protein molecule. Of these we have estimated only the first two, so that when we speak of "total carbohydrate" we mean "total free carbohydrate". Of the third fraction, attached to protein, it has been generally assumed that it is absent or negligible in the yolk of chick eggs, and was in fact thought to be exclusively a part of the mucins and mucoids, which in their turn are thought to be essentially confined to the albumen. It is now known, however, to be present in all the fractions of albumen (21) and its presence in chick egg yolk has been reported at least once (6). The complex appears to be made up of four molecules of mannose, two molecules of glucosamine and an unidentified compound of nitrogen (17). (Neuberger (17) found that the carbohydrate could not be split off from the protein by any physical means such as recrystallization, ultra-filtration, denaturation with acid, or denaturation with heat, so that the method used by us would not yield any of it.) Nothing is known of the proteins inside a salmon egg. We are of the opinion that any combined carbohydrate present is not in sufficient quantity to account for the observed rise in free carbohydrate (Fig. 1), although we have no proof of this for the

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salmon. In the frog, however, only 10 to 15% of the carbohydrate is in the combined form (4), while in the chick the combined carbohydrate makes up one-third of the total, and nearly all of it must be in the albumen (13, 14). The only salmon egg protein which has been investigated is that of the shell or casing, in which Young and Inman (22) found that glucosamine made up 1.04% of the dry weight. This quantity would be negligible for embryonic use even if it were passed over to the embryo, a point on which there is no evidence.

Methods

Several thousand recently fertilized, and a smaller number of unfertilized, eggs of the Atlantic salmon, *Salmo salar*, were obtained from the Government Fish Hatchery at Bedford, N.S., at the time when the eggs were being fertilized in November of 1937 and 1938. The fertilized eggs were reared in a hatchery at the University at an average temperature of 7° C., and determinations of the glucose and glycogen content of eggs or larvae were made weekly. Carbohydrate analyses of detached yolks, embryos, and livers were also periodically made.

The sum of the carbohydrate present in the detached embryo and yolk was less than that obtained from an analysis of the whole larva. This was to be expected since the detachment of the yolk sac caused the rupture of all blood vessels connecting the embryo with the yolk, which necessarily must have resulted in a slight loss of blood. The error for glycogen was not great, but the glucose loss was sometimes 10 to 20%. However, analyses of whole larvae were usually made at the time that carbohydrate determinations of egg fractions were carried out, and Table I has been constructed from data of both kinds.

It was found that the wet weight of eggs varies from year to year; in 1937 the average weight of recently fertilized eggs was 116 mg.; in 1938 the weight was 149.75 mg. Hayes and Ross (8) encountered a like variation in weights. They therefore invented a standard fertilized egg of 130 mg. wet weight and adjusted their figures on this basis. A similar procedure was followed in this investigation.

In the results to follow, ages have been expressed as the number of days before or after hatching. Introduced by Allen (1), this method of expressing the age of the salmon eggs and larvae decreases the error caused by varying developmental rate from year to year due to temperature variations of the water in the aquarium. The date on which most eggs hatched was taken to be the zero day or central date of hatching.

The carbohydrates present were obtained by Soxhlet extraction with water carried out for 3 to 5 hr. Material to be extracted was first placed in boiling water to destroy any enzymes capable of converting glycogen to glucose (12, p. 185). The number of eggs or larvae used in a test varied from 20 to 40 depending on the carbohydrate content.

Because of the solubility of glycogen in water (7) it was possible to estimate both glycogen and glucose from a single sample. Each extract was divided

into two portions. On one the glucose was estimated directly; in the other the glycogen was converted to glucose by acid hydrolysis before the glucose determination. The glucose value of the second portion, minus that of the first portion, multiplied by 0.927 gave the glycogen concentration (16). The customary factor for conversion of glucose to glycogen is 0.927, although Kerly (9) recommended 0.957, while Kerr (10) found the factor in acid hydrolysis to be 0.938.

Sugar was estimated by the method of Patterson (18), whose procedure was slightly modified by the use of 2 ml. of aqueous extract instead of 0.2 ml. By performing the experiment with standard glucose solutions a series of values was obtained from which a graph similar to that given by Patterson was constructed. The method is basically that of Hagedorn and Jensen, from which it differs only in the manner of determining the amount of reduced ferricyanide.

The Carbohydrate Content of Eggs and Whole Larvae

Determinations were made from the day of fertilization until the yolk sac had completely disappeared, 92 days after hatching. The results are indicated in Fig. 1 and in Table I. Glucose plus glycogen equals total carbohydrate.

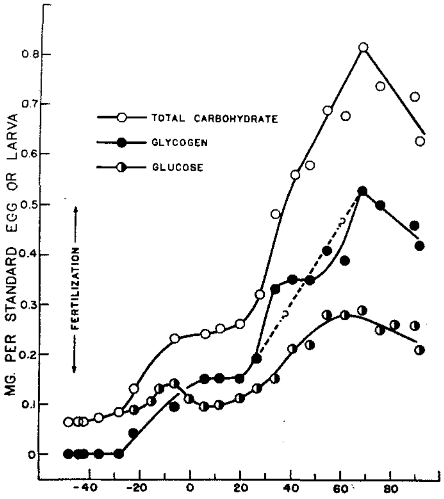

FIG. 1. *Distribution of carbohydrate in the whole egg or larva.*

TABLE I

RESULTS OF ANALYSES OF SALMON EGGS, LARVAE, AND CONSTITUENT PARTS OF THE SYSTEM

All figures are in milligrams per egg, assuming that an egg weighs 130 mg. in the early stages

1 Days before and after hatching	2 Yolk sac glucose	3 Embryo glucose	4 Total glucose	5 Embryo or total glycogen	6 Total carbo-hydrate	7 Liver glucose	8 Liver glycogen
Unfert. eggs			0.064	0.000	0.064		
−44			0.065	0.000	0.065		
−42			0.066	0.000	0.066		
−36			0.071	0.000	0.071		
−28			0.083	0.000	0.083		
−22			0.089	0.041	0.13		
−15			0.104				
−12	0.10	0.028	0.13				
− 6			0.14	0.094	0.23		
0	0.085	0.025	0.11				
6	0.062	0.033	0.095	0.15	0.24	0.004	0.01
12			0.099	0.15	0.25		
20	0.051	0.045	0.11	0.15	0.26		
27			0.13	0.19	0.32	0.0027	0.0073
34	0.053	0.084	0.15	0.33	0.48		
41			0.21	0.35	0.56	0.0028	0.04
48	0.07	0.13	0.22	0.35	0.58		
55			0.28	0.41	0.69		
62	0.10	0.14	0.28	0.39	0.68	0.0043	0.10
69			0.29	0.53	0.82		
76	0.083	0.14	0.25	0.50	0.74	0.0062	0.15
82			0.26				
90			0.26	0.46	0.72	0.009	0.22
92			0.21	0.42	0.63		
Yolk sac gone							

The carbohydrate content of eggs two days after fertilization was 0.065 mg. This was all in the form of free sugar; no glycogen was found until three weeks after fertilization. The amount of glucose in unfertilized eggs was estimated to be 0.064 mg. These determinations were made on four batches of eggs varying from 25 to 35 in number. The first two batches contained an average of 0.063 mg. of glucose, while an average of 0.064 mg. was present in the second two. Duplicate analyses were nearly always made of whole eggs. Since all glycogen in advanced stages was in embryos only, therefore the same was assumed to be true in the early stages when the embryo could not be dissected from the yolk sac. This assumption gives the early points in the embryonic glycogen curve as drawn (Fig. 3).

Except during the period of hatching, there is an increase in carbohydrate from the time of fertilization up to about 70 days after hatching when nearly all the yolk has been absorbed. The maximum concentration is 0.82 mg. per larva. There was a continued decrease in carbohydrate after this time.

A close parallel was observed between increase in glucose and in glycogen in the complete larval system, though the maximum concentration of glycogen is reached a few days after that of glucose.

From a week before hatching to a week after it there is a diminution in glucose. There is, however, no decrease in glycogen, although the curve flattens for three weeks after hatching, following which there is an uninterrupted synthesis of glycogen up to about 70 days, after which it declines.

It is notable that Hayes and Ross (8) found a drop in fat content of the embryo at hatching. Moreover Saunderson (19) found that cartilage formation in the salmon head ceased abruptly at the time of hatching. A comparison of the cartilage in the head and the carbohydrate in the larva is made in Fig. 4. It will be seen that the synthesis of carbohydrate ceases abruptly before hatching and is resumed about three weeks later. In cartilage formation also the curve flattens out for nearly three weeks, after which there is a rapid rise until it reaches a maximum. The similarity of the curves lends support to the suggestion that at hatching or birth there is a pause in the building of new structures, and in the synthesis of new materials. We do not mean to suggest by Fig. 4 that carbohydrate and cartilage have a causal relation to one another, but we do suggest that carbohydrate or fat are morphological entities in the embryo, subject to the same developmental laws as cartilage or any other tissue or organ.

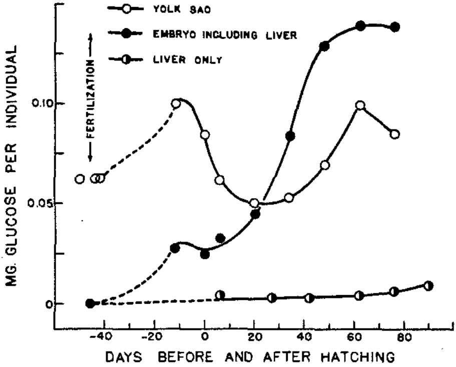

FIG. 2. *Distribution of glucose among the parts of the system.*

From a further examination of Fig. 1 it is evident that the mechanism for the building up of glucose is present from the beginning, since there is an increase in glucose a few days after fertilization. Analyses for glycogen, however, gave negative results until 24 days after fertilization. In duplicate tests a week before this no glycogen was found. This suggests that the mechanism for the synthesis of glycogen is not present in the egg soon after fertilization, but appears somewhat suddenly about three weeks before hatching.

Carbohydrate in Embryos

Determinations of the amount of glucose and glycogen present in the embryos (yolks detached but livers present) were made 12 days before hatching. This was the earliest time at which it was found possible to detach sufficient embryos to make an analysis. After that time the carbohydrate content of embryos was measured about every two weeks. It will be seen from Fig. 2 that glucose rises steadily, sloping off somewhat at hatching, following which it increases fairly rapidly and uniformly until some 60 days after hatching. A similar trend is noted for glycogen (Fig. 1) but the peak is reached 12 days later. It should be noted that "embryonic glycogen" is the same as "total larval glycogen", since all the glycogen is in the embryo.

We have been uncertain what value to give to the apparent flattening of the glycogen points between 30 and 50 days (Fig. 1), so we have drawn the curve both to show the flattening (solid line) and to suppress it (dotted line). In Fig. 3 the "embryo minus liver" curve is derived by subtraction of liver glycogen from the curve in Fig. 1, and in Fig. 3 the two glycogen possibilities are shown between 20 and 70 days.

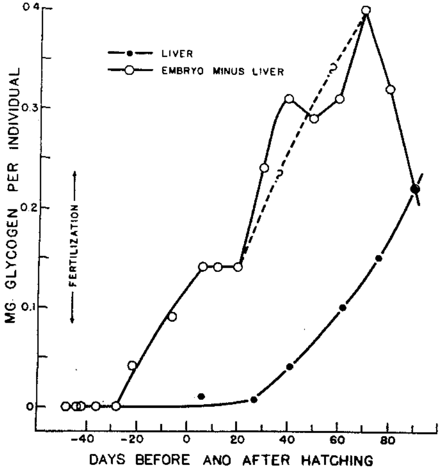

FIG. 3. *Distribution of glycogen between the liver and the rest of the embryo. The sum of these two curves appears in Fig. 1 as glycogen in the whole egg. Values for "embryo minus liver" have been obtained by reading from the Fig. 1 glycogen curve, and subtracting the liver curve. In the period from 20 to 70 days, the solid and dotted lines correspond respectively to the same region of the glycogen curve in Fig. 1.*

Detached Yolk Sacs

The carbohydrate in the yolk was estimated at the time that analyses were made of the embryos. The values are given in Fig. 2. The glucose in the yolk reaches a peak twice during larval development: about a week before hatching, and at 62 days when ordinarily the larvae would probably be feeding. As already stated, the earliest analyses of detached embryos and yolks were made 12 days before hatching. The curves in Fig. 2 to the left of −12 days have been drawn in dotted lines, their shape being based on the following evidence: at fertilization all the 0.65 mg. of egg glucose must have been in the yolk, since there was no embryo. Similarly, the embryonic glucose at fertilization was zero. Between fertilization and −12 days the sum of the embryonic and yolk curves must give the values in Table I, column 4.

When the last analyses were made the yolk was almost completely absorbed, and the sac could be detached only with great difficulty. At the time of the final analysis of larvae at 92 days the yolk sac had completely disappeared.

No glycogen was detected in the yolk sac except on two occasions, and then in such minute quantities—of the order of 0.01 mg.—that it might well be attributed to error. Therefore, all the glycogen present in the larval system was stored in the embryo.

Livers

Periodic estimations were made of the carbohydrate content of the liver. The results are shown in Figs. 2 and 3. Only minute quantities of reducing sugar were found. An examination of the graph indicates that the liver

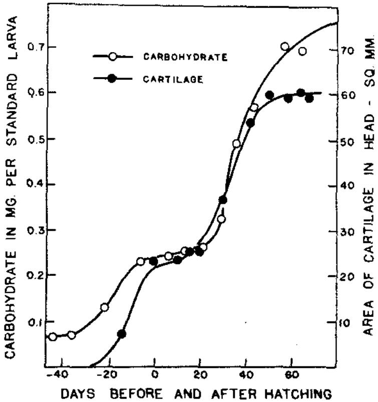

FIG. 4. *A comparison of the total carbohydrate in a developing salmon larva with the area of cartilage in the head. Cartilage values are from Saunderson (19).*

does not act as a storage place for glycogen until some 30 to 40 days after hatching, when this function is rather suddenly assumed. After this time the glycogen content rapidly increased until the last analysis was made 90 days after hatching, when 0.222 mg. were stored there. This rapid rise in glycogen will be discussed later.

Discussion

From Fig. 2 it will be observed that the embryonic and yolk glucose tend to rise and fall together. The simplest explanation of this is found in the suggestion that glucose in an embryo, as elsewhere, is not stored in any specific tissue, but passes freely through all walls and is distributed throughout the larval system as an aqueous solution equally concentrated in all parts. According to this interpretation the ratio of glucose between embryo and yolk at any time serves merely as an index of the relative quantities of water in each. It also follows that at any time, the embryo with 80 to 90% water will contain more glucose per gram wet weight than the yolk with 50 to 60% water.

Fig. 2 shows that the synthesis of glucose from some source (not glycogen) begins at the time of fertilization. It is presumably accomplished by means of enzymes within the embryonic cells and will therefore be proportional in intensity to the number of such cells. This synthesis of glucose never stops during development, although it is quite possible that its rate diminishes for a short time before hatching. Against this continual synthesis we have to set *(a)* the utilization of glucose as fuel, which undoubtedly goes on all the time although we cannot express it quantitatively, and *(b)* the removal of glucose as glycogen. The capacity for glycogen formation is not, like that of glucose, present from the beginning, but appears rather suddenly some 28 days before hatching. Histochemical studies showed that the place of storage was chiefly the muscles; those of the caudal and dorsal fins stained markedly with 0.1 *N* iodine solution. Probably the onset of glycogen storage corresponds to the time in embryonic development when muscle tissue becomes differentiated. Glycogen storage by the liver is discussed below, but it may be noted here that during the whole larval period muscle storage is much the more important part, an observation which points to the danger of interpreting embryonic glycogen as a demonstration of liver storage.

In Fig. 1 a rather constant time lag between glucose and glycogen fluctuations will be noticed. Thus glucose begins to diminish some eight days before hatching, and two weeks later the synthesis of glycogen stops. Six days after hatching glucose is synthesized again, and two weeks later the same thing happens to glycogen. At 60 days the glucose curve again turns down, and again 10 days later the glycogen curve follows. This last change happens in spite of the fact that the liver glycogen pursues its course unaltered. The suggestion comes very readily that we are dealing in the muscle cells (though not in the liver) with a reaction

$$\text{Glucose} \rightleftharpoons \text{Glycogen}$$

which may proceed in either direction depending on the relative concentrations of the two substances. The embryo is therefore taking any of the new glucose that is left over after metabolic needs are satisfied and converting it into muscle glycogen. In time of need this muscle glycogen is drawn on as a reserve. The final drops in all three curves after about 70 days are to be interpreted as the onset of starvation, for at this time the yolk is almost used up. By the end of our observations at 92 days there was no yolk sac visible at all.

It is interesting to find that Shaw (20) noted the disappearance of glycogen from the livers of chicks on the day of hatching, although glycogen was present in the liver the day before and two days after hatching.

One of the most striking things in these analyses is the sudden appearance of glycogen in the liver some 40 days after hatching, and its rapid rise after this time. Fig. 3 represents the glycogen in the liver and in the embryo minus liver. The curve for the embryo without liver rises steadily until the 68th day, and then declines sharply. This corresponds to the time when the storage of glycogen by the liver is taking place most rapidly. The yolk has not yet been completely absorbed, and there is still an increase in total embryonic glycogen.

The sudden glycogenic function of the liver has been noted by many investigators, and appears to be almost universal among the vertebrates. Lochhead and Cramer (11) found that though definite amounts of glycogen were present in foetal livers of rabbits, the percentage was very low up to the 25th day. This represented the time when the liver assumed its adult glycogenic function. They noted that this coincided with the beginning of the decrease in placental glycogen.

In studies on various embryos, Aron (2) showed that in the pig the liver functioned as a storage place for glycogen near the end of gestation. In the sheep, glycogen is found in appreciable quantities at the end of the fourth month of pregnancy, and in the guinea pig the glycogenic function is assumed more slowly but definitely in the last four days of gestation. He discussed the appearance of the islets of Langerhans out of the islets of Laguesse, and found a striking coincidence between the appearance of the islets of Langerhans in the pancreas and the deposition of large quantities of glycogen in the liver. Moreover, he endeavoured to show that the islets of Langerhans produce insulin, but those of Laguesse do not. From further investigations by histochemical methods Aron (3) found no glycogen in the liver before the appearance of the hind limb buds, and that removal of the pancreas in early stages prevented the appearance of glycogen in the liver. As a result of his research, Aron concluded that the beginning of this function of the liver varies from species to species but manifests itself at a fixed time in ontogeny. The present work supports his theory.

There is, of course, no pancreas in teleosts, but in the adults there are well-defined masses representing the islets of Langerhans. Whether the appearance of glycogen in the liver of the salmon is related to islet tissue must remain for

the present a matter of speculation, since no reference has been found to the time of its appearance.

There is a synthesis of carbohydrate from fertilization, uninterrupted except at the time of hatching, until the beginning of the final starvation period. We have examined the literature but can find no other case in which the embryo ends up with more carbohydrate than the egg had at the beginning. This unique property of the salmon egg is no doubt shared by other teleosts. We do not know what the source of this new carbohydrate is. Certainly the great period of carbohydrate synthesis after hatching corresponds to a sharp drop in fat (8), although, since the carbohydrate gain amounts to only one-tenth the fat loss, the two cannot be said to be quantitatively related. For the three weeks before hatching there is a synthesis of both fat and carbohydrate, although the absolute quantity of carbohydrate involved is quite small, of the order of 0.1 mg. per egg. This gain might be accounted for by the combined carbohydrate in protein molecules or by the protein itself.

A synthesis of glycogen (as distinct from total carbohydrate) is found in the chick as well as in the salmon (13). In the salmon the glycogen is all in the embryo. In the chick, however, Needham divided the system into "embryo" and "remainder of the egg". The latter part included not only the yolk and albumen, but the allantois, amnion, and the coelom with its two walls each composed of two germ layers. It is not surprising therefore that the newly formed glycogen was found in both parts. Needham does not suggest, nor do we see any evidence for, the storage of glycogen in the yolk or albumen. Its storage as in the salmon is evidently cellular, the extra embryonic portion being laid down in the blastoderm. In our method of separating the yolk sac from the salmon embryo, the division was made in the coelom, so that the somatic mesoderm and ectoderm were included with the embryo, the splanchnic mesoderm and endoderm with the yolk sac. The quantity of cellular material which went with the yolk sac was a very small proportion of the whole embryo. The glycogenic function of the extra embryonic membranes of the chick is probably something new which has arisen with the membranes themselves.

The view has been expressed that glucose traverses cell walls and is found everywhere in the salmon system, including the yolk. Glycogen on the other hand is evidently confined to cells. If the enzymes necessary to synthesize glycogen act only in cells, it is, to say the least, probable that the enzymes which promote the production of glucose from glycogen also act in cells. They may very well be the same enzymes. With this distinction between glucose and glycogen in mind, we may examine Table II, which shows the stores of carbohydrate with which different eggs start out. So far as investigated, eggs of birds, reptiles, and fishes, have their initial store as glucose, with little or no glycogen. Amphibia and invertebrates on the contrary have stores of glycogen with little or no glucose. The only exception is the trout egg, in which Fauré-Fremiet and Garrault report 0.34% glycogen. About the same time as the trout work, however, Fauré-Fremiet and his collaborators were

TABLE II

THE CONCENTRATIONS OF GLUCOSE AND GLYCOGEN IN VARIOUS EGGS AT THE TIME OF FERTILIZATION

All figures are in per cent wet weight

Animal	Glucose	Glycogen	Author
Chick, whole egg	0.20	0.003	Various authors
Chick white	0.50	0.003	Sakuragi
Chick yolk	0.33	0.002	Sakuragi
Tortoise	0.14		Diamare
Frog		2.5	Various authors
Herring	0.50		Steudel and Osato
King salmon	0.096		Greene
Atlantic salmon	0.050	0.000	This paper
Trout		0.34	Fauré-Fremiet and Garrault
Starfish		0.020	Dalcq
Sea urchin *(Echinus)*		1.3	Moore, Whitley, and Adams
Sea urchin *(Strongylocentrotus)*	0.08	1.28	Ephrussi and Rapkine (5)
Silk worm		1.5	Various authors
Bee		2.5	Straus
Sabellaria (Polychaet worm)		1.3	Fauré-Fremiet

NOTE.—*Chick figures are from Needham (15, Vol. 1, p. 278), other figures are from Needham (15, Vol. 1, p. 356), except Strongylocentrotus (for which see (5)) and our salmon figures.*

reporting glycogen concentrations in frog eggs much greater than those found by others—sometimes four or five times as high as the average of other workers. For this reason we are inclined, pending confirmation, to disregard the trout figure.

If we look for an underlying morphological explanation for Table II, we find that the "glycogen" eggs are those in which the whole egg divides into cells that participate in development. In the frog, for instance, though the cells in early development differ markedly as to size, each cell will contain a store of glycogen. One does not think of the embryo as taking material from the yolk, but rather of the yolk cells continuing to divide and changing gradually into differentiated embryonic cells. The stores are therefore divided from the outset.* There is a time rather early in development when most of the activity is in small cells with limited stores, and conversely, most of the stores are in the relatively inactive yolk cells. It is during this time that the active cells, with the glycogen store (as we think) locked away from them, use up most of the available combined carbohydrate (4). Later the problem solves itself by cell division, which breaks up the storage cells and distributes them; and by the acquisition with continued differentiation, of glycogen-splitting enzymes in the former yolk cells. The same argument applies with even fewer provisos to the eggs of echinoderms, while in the insects the nucleus continues to divide without cell walls until the several hundred nuclei migrate to the surface and cell walls are formed. All the egg material

* *The division of stores is clearly illustrated by unpublished microphotographs of Amblystoma larvae which Dr. Herbert Coar has kindly shown us. Here the muscle cells, after the differentiation of fibres and cross striations, still contain numerous yolk platelets.*

therefore participates in early development. The polychaetes have total spiral cleavage, so that again there is nothing corresponding to a yolk sac.

Turning to the "glucose" eggs of birds, reptiles and teleosts, it is found that the embryonic stores are locked away in the large, non-cellular yolk sac. As the small embryo uses up glucose it is replaced by simple diffusion from the yolk, a mechanism that would not serve if glycogen made up the carbohydrate store. The glucose taken by the embryo is constantly replaced and augmented, although we do not know where or how. Now if we were to take a salmon egg, which is about half water at the beginning, and by the addition of water to make it two and one-half times as heavy as it was before, it is obvious that there would be four times as much water as before, and that if the glucose per egg remained the same, the glucose tension would drop to one-quarter its former value. If at the same time as the water, we added enough glucose to make a fourfold increase per egg, then the glucose tension would remain unchanged. In a salmon larva at the end of the yolk sac period there actually is four times as much water, and as Table I, column 4 shows, four times as much glucose, as well. We are inclined to conclude that the glucose tension throughout development remains the same, and that, as already mentioned, when glucose tends to exceed the fixed level glycogen is formed, and vice versa. It will be possible to formulate this theory quantitatively when we have a good set of wet and dry weights. At first sight the chick appears to develop in the opposite manner, since there is a diminution in glucose per egg during development, but it must be remembered that the chick gets drier, not wetter as it develops, and moreover the part played by the amniotic fluid with respect to glucose is not known. It is quite possible that the glucose tension in terms of embryonic chick water is constant throughout development.

In conclusion mention may be made of the rate at which new carbohydrate is formed in the salmon. We cannot do this quantitatively, but an inspection of Fig. 1 shows that the curve for total carbohydrate is steepest in the periods −28 to −6 and 24 to 45 days. These probably correspond to the periods of maximum synthesis per unit wet or dry weight of embryo. The first of these fits very well with the table of anticipated energy sources previously published (8, p. 372). The second maximum, however, is something new and we do not at the moment see that it has any correspondence in chick embryology.

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PARASITES OF FRESHWATER FISH

II. PARASITISM OF SPECKLED AND LAKE TROUT AND THE FISH FOUND ASSOCIATED WITH THEM IN LAKE COMMANDANT, QUE.[1]

BY L. L. LYSTER[2]

Abstract

Parasitism of two game fish, *Salvelinus fontinalis* and *Cristivomer naymaycush* and of two other fish, *Perca flavescens* and *Catostomus commersonii* is discussed. *Glaridacris intermedius* sp. nov. (Cestoda), *Rhabdochona laurentiana* sp. nov. (Nematoda) and *Raphidascaris alius* sp. nov. (Nematoda) are described.

In presenting further information on parasitism in freshwater fish in the Province of Quebec it is hoped that a contribution will be made not only to distribution records, but also to the interrelation of parasitic forms. The first paper of this series (13) dealt with general trematode parasites. Collecting was done over an extended period and in a considerable area, and although several trematodes were found in two or more hosts, it was impossible to judge what part any fish might play in maintaining an infection in another. The present paper, on the other hand, is based on material collected in limited and known waters and within a short period of time. This material was collected during the months of September and October, 1938, in Cameron's Bay, a southerly projection of Lake Commandant.

Four hosts are considered, *Salvelinus fontinalis*, *Perca flavescens*, *Catostomus commersonii*, and *Cristivomer naymaycush*. Of these, the first three, speckled trout, yellow perch, and common sucker, were invariably found together. The fourth, lake trout, was never found in association with the other fish, but was taken in the deeper parts of the same waters; it was included in the survey in the hope that its parasite records might indicate whether identical parasitism was more apt to occur among totally unrelated fish of similar habitat, or related fish living under different conditions.

TREMATODES

Two species of *Crepidostomum* were collected, one from perch and one from speckled trout. In addition a trematode reported as a new species from perch in the first paper of this series was found in small numbers in the perch and in considerable quantity in the trout. Both suckers and lake trout were free of trematodes. Metacercariae of a member of the genus *Apophallus* were present in large numbers in the skin of speckled trout, and metacercariae of a strigeid were noticed in small numbers in three of the perch. These immature forms will be discussed later.

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[2] *Graduate assistant.*

Crepidostomum isostomum Hopkins, 1931

HOST: *Perca flavescens*

Material: three specimens

These hexapapillate forms have features that suggest identification as either *Crepidostomum isostomum* Hopkins 1931, or *C. canadense* Hopkins, 1931. Measurements of major structures are very similar to those of *C. isostomum*, but are consistently greater, and uniformly twice those for *C. canadense*, except that of width. The ratio, length of pharynx to diameter of oral sucker, the shape of the ovary and its position in relation to the ventral sucker are different from *C. isostomum* but similar to *C. canadense*. The post-testicular condition of the vitellaria is unlike that described for *C. canadense* but like *C. isostomum*. With these exceptions, all considered variable, the present material could be assigned to either of the species. *C. isostomum* and *C. canadense* are thought by their author to be closely related (8), and since this material seems to possess characters of both species, *C. canadense* has been listed as a synonym of *C. isostomum*.

Three of these trematodes were recovered from a single yellow perch from the lake. They were long, narrow forms 2.5 by 0.575 mm., widest and deepest in the ovarian region, tapering to a point terminally and narrowing to a neck posterior to the prominent hexapapillate oral sucker. Oral sucker and acetabulum uniformly round, 0.25 mm. in diameter. Reduced prepharynx. Pharynx slightly longer than broad, 0.074 by 0.098 mm. Oesophagus long, opening in caeca dorsal to acetabulum. Caeca terminating behind testes. Cirrus sac a short wide structure curving slightly dorsally but only slightly exceeding or not quite reaching anterior margin of acetabulum; 0.25 mm. in greatest measurement, opening into common genital pore anterior to intestinal bifurcation. Ovary lateral, globular, removed posteriorly from acetabulum by about its own diameter, 0.24 mm. Globular seminal vesicle contiguous with posterior margin of acetabulum to dorsal of ovary, slightly larger than that organ. Testes irregularly shaped, margin entire, medial, slightly oblique and posterior to ovary and receptacle, about 0.28 mm. in diameter; considerably removed from posterior end of body, close together but not contiguous. Vitellaria extend from cirrus sac to posterior end of body in lateral medial area, few follicles dorsally and meeting posteriorly but not overlapping behind testes. Uterus with few small eggs, convoluted in region of ovary and looping to anterior margin of anterior testis.

Crepidostomum cooperi Hopkins, 1931

HOST: *Salvelinus fontinalis*

Material: 50 specimens

The anterior extent of the vitellaria, the relative size of the pharynx and suckers, and the posterior position of the crural furcation are confusing details in these forms, as well as the comparatively large size of the eggs. The body size, the vitellaria, and general organization are characteristic of *C. cooperi*,

particularly the variant described as *C. fausti* by Hunninen and Hunter (10). The absolute measurements of the sucker and egg are suggestive of *C. farionis*, but, in proportion to body size, they are larger. The fact that the body is recurved and somewhat contracted (but not shrunken) anterior to the acetabulum, would perhaps explain the rather posterior position of the crural furcation and the more anterior genital opening than is typical, as well as the extensive vitellaria in this region. This condition and the extreme variability of the species will not allow for a diagnostic importance for these atypical details, and the form has, therefore, been listed as *C. cooperi* Hopkins, 1931.

They are compact, broad, thick forms, 0.70 to 1.49 by 0.25 to 0.56 mm., body widest at the level of the oral sucker or at the acetabulum, or sides of body almost parallel and the ends bluntly rounded, thickest at the posterior margin of the acetabulum, and usually ventrally curved. Oral sucker subterminal 0.11 to 0.21 by 0.14 to 0.25 mm. with six non-prominent papillae rarely extending past limits of the body. Pharynx globular, about 0.08 to 0.015 mm. in diameter, dorsal and posterior to oral sucker, prepharynx, and pharyngeal vestibule. Oesophagus short, bifurcation at anterior margin or slightly in front of it. Crura extending well posterior to testes. Genital pore ventral to intestinal furcation. Cirrus sac long, slender, widest posteriorly passing dorsally to acetabulum to its posterior margin or slightly beyond, never filled by seminal vesicle. Acetabulum larger than oral sucker, 0.14 to 0.28 by 0.17 to 0.35 mm., greatest dimension laterally, medial, well anterior, and often almost in contact with oral sucker. Ovary globular, slightly lateral, dorsal of posterior margin of acetabulum, or slightly more posterior, 0.7 to 0.14 mm. Seminal receptacle varying in shape and size, and medial or submedial, not far behind ovary. Testes two, tandem, and globular or slightly wider than long, margins entire, 0.08 to 0.17 by 0.15 by 0.18 mm. removed by at least one diameter, and usually more, from terminal area of body. Uterus with many large intra-uterine eggs about 56 by 84μ extends as far as midpart of posterior testis. Vitellaria of large closely packed follicles filling all available space from anterior to posterior limits of body but somewhat limited dorsally. Excretory bladder obscured by these structures.

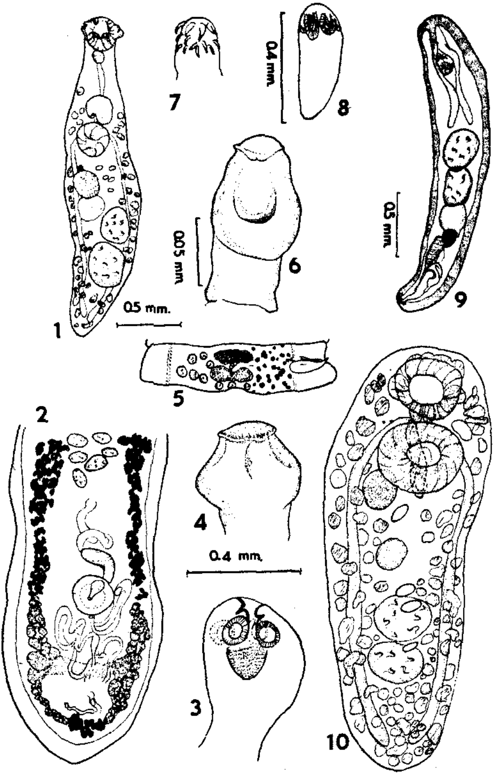
This species has already been reported from this host in Quebec by Richardson (15) under the synonymous name, *C. fausti* Hunninen and Hunter, 1933.

Ptychogonimus fontanus Lyster, 1939

HOSTS: *Perca flavescens, Salvelinus fontinalis*

Material: 25 specimens

This species was established in the first paper of this series from limited material collected from a perch (13). Many specimens were since collected from *Salvelinus fontinalis*, in the stomach and upper digestive tract. Little can be added to the original description of the species. In the present material it is noted that an oesophageal structure is present as a short, weak, saccate body, opening dorsally from the pharynx, and leading laterally and



FIGS. 1–10. *Trematodes, Cestodes, and Acanthocephala.* 1. *Crepidostomum isostomum from Perca flavescens.* 2. *Glaridacris intermedius sp. nov. posterior end, from Catostomus commersonii.* 3. *Ichthyotaenid from S. fontinalis.* 4. *Glaridacris intermedius sp. nov. anterior end, from C. commersonii.* 5. *Eubothrium salvelini mature proglottid from S. fontinalis.* 6. *Eubothrium salvelini scolex from S. fontinalis.* 7. *Neoechinorhynchus cylindratus—proboscis from S. fontinalis.* 8. *Ichthyotaenid from Cristivomer naymaycush.* 9. *Neoechinorhynchus cylindratus, entire worm from S. fontinalis.* 10. *Crepidostomum cooperi from S. fontinalis.*

anteriorly into the crura, a condition similar to that noticed in the related genus *Hirudinella* (4). The crura are naturally convoluted and much longer than the body cavity. This is uniform in all specimens and not due to contractions as previously suggested.

Cestodes

Polyzootic cestodes were limited to the trout. Adults of a single species and immature forms of this genus and another were found in *Salvelinus*. Similar immature cestodes were taken from the lake trout. Monozootic cestodes of a hitherto undescribed species were recovered from the common sucker. Perch carried no cestode infections. By far the heaviest parasitism noted was that of the polyzootic cestodes in speckled trout. All specimens of this fish carried some of the parasites and most had large numbers.

Eubothrium salvelini (Schrank, 1790)

Host: *Salvelinus fontinalis*

Material: 30 specimens

Presence of non-armed and non-specialized pseudobothridia, terminal disc, lateral genital opening, and saccate uterus show they belong to the genus *Eubothrium*. The shape of the scolex, length of the cirrus sac, and organization of vitellaria refer them to the species *E. salvelini* (Schrank, 1790), following the system of classification of Joyeux and Baer (12).

Scolex longer than wide, widest behind base of prominent pseudobothridia, and bearing bilobed terminal disc. Proglottids wider than long, about 1.68 by 0.7 mm. or greater, and considerably greater when compressed, always wider than scolex and often secondarily divided by transverse fissures. Genital opening lateral, alternating only rarely. Ovary bilobed, posterior central uterus saccate, anterior, central. Testes numerous, more or less spherical, about 0.07 mm. in diameter, occupying lateral intermuscular field and contiguous in neighbouring segments. Cirrus sac about 0.07 mm. at widest point in region of longitudinal nerve, about 0.6 mm. long. Cirrus broad, leaf-like when extruded, with minute spines. Vitellarian follicles small, scattered in central lateral fields. Vagina opening posterior but close to male genital openings.

Glaridacris intermedius sp. nov.

Host: *Catostomus commersonii*

Material: 10 adults

Four species have been listed by Hunter (11) in this genus of monozootic cestodes. Of these, two species, *G. hexacotyle* Linton, 1887 and *G. catostomi* Cooper, 1920, do not possess a terminal disc, while two species, *G. confusus* Hunter, 1929 and *G. laruei* Lamont, 1921, like the present form, have this structure in common, as well as non-operculate eggs and the absence of a genital atrium. Like *G. confusus*, this form has a granular ovary, not distinctly separate from the vitellaria, similar body shape and testes in two

lateral rows. Like *G. laruei* it has 70 testes and the vitellaria tend to enclose them. Measurements, egg size, etc., are much alike in *G. confusus*, *G. laruei* and the present form.

The significance of these similarities and differences is obscure at this time, and may indicate synonymities. The present form is apparently closely related to *G. confusus* and *G. laruei*, but cannot be referred to either. It must, therefore, be assigned to a new species which has been designated *G. intermedius* sp. nov.

The adults range in size from 3.5 to 9.0 mm. in length. They are widest at the level of the cirrus sac, 0.5 to 0.84 mm., and taper terminally to a rounded point, anteriorly to the region of the origin of the vitellaria, 0.4 to 0.55 mm. wide, which follows the narrower neck. The scolex is well differentiated and short, rounded at the junction with the neck, and squared anteriorly; it has three pairs of loculli and a terminal disc. All organs are entirely medullary. Vitellaria originate about 0.35 to 0.84 mm. behind the base of the scolex, extending as far as the ovary and with post-ovarian follicles. The testes are about 70 in number, arranged in two more or less parallel rows from a point slightly behind the origin of the vitellaria to about 0.30 mm. anterior to the cirrus sac; they are irregularly oval in shape, 0.07 by 0.14 to 0.07 by 0.084 mm. greatest diameter transversely. The vitellaria occur chiefly in the lateral areas, but scattered follicles are dorsal to the testes. The vas deferens is highly convoluted and enters a short, curved seminal vesicle dorsal and anterior to the cirrus sac. Cirrus sac small, circular, 0.084 to 0.085 mm. in diameter. Common genital atrium absent. H-shaped ovary varies in size, but wings usually about 0.5 to 0.7 mm. long; lateral branches relatively narrow, medial branch also limited. This organ is granular, finely lobate, similar in appearance to and in contact with lateral and terminal vitellaria. Uterus long, convolute, reaching to posterior extremity of body, and anteriorly to the cirrus sac; vagina medial, ventral. Seminal vesicle not obvious. Female system opens close to posterior edge of cirrus sac. Eggs non-operculate, about 42 by 28μ.

Larval Cestodes

Larval cestodes of two recognizable types were taken from both species of trout. Plerocercoid forms of *Eubothrium* were found in *Cristivomer namaycush*. These consisted of a scolex and a short undifferentiated body. It is not possible to suggest the species to which these might belong, or to offer any details of their organization. The rather short scolex and deep pseudobothridia might be associated with *E. salvelini*. In other larval forms from this host four muscular suckers were present. These specimens seemed of two types. One consisted of a simple, saccate structure with four incomplete suckers alone distinguishable. In the other a fifth glandular structure was present; this type was also found in the speckled trout. These probably are referable to the family Ichthyotaeniidae Ariola, 1899 (Syn. Proteocephalidae Larue, 1911) as discussed by Joyeux and Baer (12).

Acanthocephala

Acanthocephalids were present only in speckled trout and suckers. Light infections in each were caused by the same species.

Neoechinorhynchus cylindratus Van Cleave, 1913

Hosts: *Salvelinus fontinalis*

Catostomus commersonii

Material: mature males and females were found in considerable numbers in the trout; one of each sex was taken from the sucker.

They were short, straight or slightly curved. Males were 3.0 to 4.9 by 0.6 to 0.82 mm., females about 5.1 by 0.82 mm. (longitudinal measurements exclusive of proboscis), broadest just posterior to the lemnisci. Proboscis longer than broad, about 0.53 by 0.12 mm., armed with three rows of six recurved hooks each. The anterior hooks were 0.4 to 0.45 mm. long, those of the two posterior rows were 0.21 to 0.03 mm. long. The hooks alternate in adjacent rows and are without collars or ornaments.

Embryos numerous and large, 0.07 by 0.10 to 0.12 mm. Lemnisci reach posteriorly almost to testis or almost half of body length. Brain located at base of proboscis receptacle. Testes closely applied, tandem, shortened cylinders, equal in size, 0.34 by 0.37 mm. Cement gland, a single body close behind testes, opening into cement reservoir. Small copulatory bursa present in males though often folded and inconspicuous. Genital openings terminal in both sexes.

Nematodes

Nematodes are represented in the collection by two species from the speckled trout and one from the sucker. Material from the latter host was adequate, but that from the trout included few specimens and those of a single sex. Identification is, therefore, only as accurate as is possible with limited material.

Lake trout and perch were nematode-free.

Cystidicloides harwoodi (Chandler, 1931)

Host: *Salvelinus fontinalis*

Material: one female; two immature males (uncertain)

This species was described as *Cystidicola harwoodi* from speckled trout by Chandler (2) and as *Metabronema canadense* by Skinker (17) from the same host. The latter author later placed it with *C. fischeri* in her new genus *Cystidicloides*, based on the nature of the post-cloacal papillae (18). This has been accepted by Van Cleave and Mueller (21), who reported it from *Salmo fario*.

The following description is based on the single female specimen.

Length 9.0 mm., greatest width 0.3 mm. Lips two, small and inconspicuous with slight chitinization at anterior corners, each bearing two papillae. Mouth simple, leading directly into tubular vestibule. Oesophagus in two distinct divisions; first part convoluted, occupying about 0.8 mm., second part 2.6 mm. long, emptying into simple intestine through a terminal sphincter. Divergent ovaries originate 0.15 mm. behind the oesophagus and just anterior to the anus; they are very convoluted. Bicornate uterus enters vagina just posterior to vulva, posterior uterine branch passes forward past this position to join vagina more or less parallel with anterior branch. At the level of the vulva the body diameter is sharply reduced by 0.112 mm. and on this projection the vulva opening is placed. Intra-uterine eggs 27 by 57μ; no terminal filaments noted. End of body bluntly rounded 0.116 mm. posterior to anus.

Two immature male forms slightly under 7.0 mm. in length and 0.14 mm. wide at the base of the oesophagus may belong to this species. The relationship is, however, not definite. The mouth and alimentary canal are essentially as in the female. The genital organs are poorly differentiated but a testis originates a short distance behind the oesophagus. The two spicules are dissimilar. The right member is short and wide, about 0.16 mm. long, pointed terminally but widening quickly to make the sides almost parallel; it is concave during the first three-quarters of its length and constricted just before the base. The left spicule is about 0.47 mm. long and narrow, with a widened base and a narrow wing rising at about the limit of the first quarter. Stalked, *not double* papillae present, four pre-cloacal and four post-cloacal. About 0.12 mm. behind the cloaca the body terminates in a blunt point. Conspicuous caudal alae extend about 0.028 mm. beyond the body margin.

Raphidascaris alius sp. nov.

HOST: *Salvelinus fontinalis*

Material: two males

The genus *Raphidascaris* is poorly organized and some of the descriptions of its species are inadequate, if not invalid. Several species have been reported from Europe and Asia and five North American species are already known. The present form cannot be assigned to any of these species. It differs widely in relative size of structures from *R. cayugensis* Wigdor, 1918 (23), *R. brachyurus* Ward and Magath, 1917 (22), and *R. anchoviellae* Chandler, 1935 (3) and, in the possession of similar alae and spicules, from *R. canadensis* Smedley, 1923 (19). It most closely resembles the trout species *R. laurentianus* Richardson, 1937 (16) in size and conformation, but the presence of lateral alae is a significant difference; Richardson's description of the species makes no mention of such structures. The same feature differentiates this form from *R. diadonus* Thwaite, 1927 (20); and the relative length of oesophagus and appendix are very different in *R. gigi* Fujita, 1928 as described by Yamaguti (24). Significant differences in size separate it from *R. acus* Bloch, 1779 (1) and *R. chirocentri* Yamaguti, 1935 (24).

Though the material is limited these differences necessitate the creation of a new species to accommodate it. This has been designated *Raphidascaris alius* sp. nov.

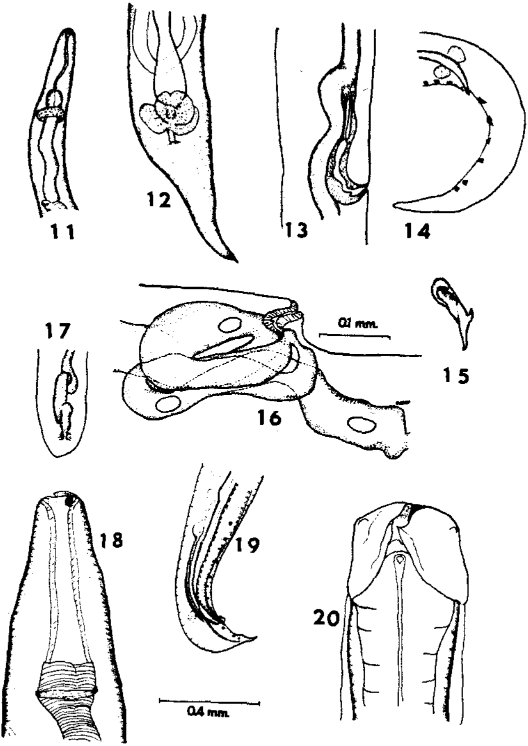
They are fairly large worms, 15.0 mm. long and 0.3 mm. wide at the base of the oesophagus and 0.4 mm. wide at the widest point. The head consists of three large lips bearing the usual papillae and conspicuous cuticular enlargements. The oesophagus is divided into a long anterior muscular portion 1.6 mm. long and a short sub-globular ventriculus 0.17 mm. long, which opens into an oesophageal appendix 1.3 mm. by 0.056 mm. and the simple intestine. The fore part of the body is curved ventrally and the tail is bent upon itself. The single testis rises just posterior to the end of the appendix, the length of the coils being greater than the width during most of its convoluted course. Just before entering the simple vas deferens, which is 0.7 mm. long, these coils are almost circular, giving a beaded appearance. Two equal and similar winged spicules are present, 0.63 mm. long and 0.028 mm. wide. They carry a terminal enlargement 0.084 by 0.112 mm. and taper to a fine point distally. The tail ends sharply 0.182 mm. behind the cloaca. On either side small pedunculate pre-cloacal papillae are present; anterior to these two or three small conspicuous elevated areas appear to mark the position of further more reduced papillae. Posterior to the cloaca three similar inconspicuous elevations probably represent the post-cloacal papillae. A conspicuous boss occupies the fore margin of the cloacal aperture. Equal lateral alae extend along the entire body length from a point just in front of the posterior extremity to the base of the lips. They are widest from the anterior limit to the region of the ventriculus.

Rhabdochona laurentiana sp. nov.

HOST: *Catostomus commersonii*

Material: 5 specimens of each sex; females non-ovigerous

This species and *R. cascadilla* Wigdor, 1918, the only other reported North American species, differ in several details, but notably in the length of the oesophagus. In Wigdor's (23) species, this structure is 1/25 the length of the body; in the present material it occupies one-quarter or more of the body length. Various differences are also apparent between it and the known European and Asiatic forms. Unlike *R. accuminata* Molin, 1860 (14) and *R. denudata* Dujardin, 1845 (5), it has a well marked pre-cloacal boss and greater numbers of papillae. Its spicules are dissimilar, while those of *R. salvelini* Fujiti, 1928, are similar (6). The terminal tail spine is not found in *R. gambiana* Gendre, 1922 (7). This form lacks the lateral teeth of *R. gymnocranii* Yamaguti, 1935, but possesses vestibular rods unlike *R. girellae* Yamaguti, 1935 (24). The extensive vestibule of *R. macrolainia* Gendre, 1922 (7), and the terminal spicular barbs of *R. zaconi* Yamaguti, 1935 (24), serve as points of differentiation, as does the position of the vulva opening in *R. amago* of this author.



FIGS. 11–20. *Nematodes.* 11. *Rhabdochona laurentiana sp. nov. anterior region female, from C. commersonii.* 12. *R. laurentiana sp. nov. posterior region female, from C. commersonii.* 13. *R. laurentiana sp. nov. vulvar region, female from C. commersonii.* 14. *R. laurentiana sp. nov. posterior end of male showing left spicule and post-cloacal papillae, from C. commersonii.* 15. *R. laurentiana sp. nov. right spicule, from C. commersonii.* 16. *Cystidicloides harwoodi, vulvar region, female, from S. fontinalis.* 17. *C. harwoodi, posterior end of female, from S. fontinalis.* 18. *C. harwoodi, anterior end of female from S. fontinalis* 19. *Raphidascaris alius sp. nov. posterior end of male from S. fontinalis.* 20. *R. alius sp. nov. anterior end of male from S. fontinalis.*

This form is, therefore, described as a new species, ***Rhabdochona laurentiana***, the first member of the genus from *Catostomus commersonii* and the first from any Canadian host.

They are cylindrical forms, attenuated anteriorly, in which the cuticle is plain and unstriated throughout. The females are about 8.3 by 0.15 mm. with a long tail terminating in a distinct chitinized spine. The males are about 5.04 by 0.13 mm. with a long tail, but no specialized point. The lips and mouth are simple. The infundibuliform vestibule is supported by longitudinal rods which are free and pointed distally; it is about 0.08 mm. long. The oesophagus is in two distinct parts; the short anterior, muscular portion is about 0.2 mm. and usually, but not invariably, somewhat convoluted; the posterior portion is about 1.8 mm. long, occupying most of the body width where it opens into the simple and narrow intestine.

Paired, opposed genital organs occur in the female. In well developed specimens the anterior ovary rises just in front of the anterior branch of the uterus, extends to the posterior end of the oesophagus or slightly further, then curves back to join the uterus. The posterior ovary rises just behind its branch of the uterus, extends back to the anus, then returns to enter the uterus. The vulva is situated about two-thirds of the body length from the anterior end. From it the vagina runs directly posterior for about 0.2 mm. to receive the two branches of the uterus. An ovejector is present.

The male is recurved terminally. Two dissimilar spicules are present. The heavy right member, about 0.13 mm. long, is concave and equipped with backward pointing projections. The left one is simple and slender, about 0.5 mm. long, and bears a narrow wing rising in the last quarter of its length. Six conspicuous post-cloacal papillae and 12 pre-cloacal papillae are present. The last pre-cloacal members are not conspicuous. In addition to these a boss bearing a papilla-like structure is present just anterior to the cloaca. The single testis rises a short distance behind the oesophagus; it is simple and unconvoluted; no differentiation seems possible between it and the vas deferens. Neither caudal nor cephalic alae are apparent.

This was the only nematode found in *Catostomus commersonii*.

Conclusions

Though definitely limited in significance, it is interesting to note the interrelated host-parasite list. Lake trout were remarkably free from parasites, while the speckled trout were the most heavily parasitized hosts. The two other fish appear to be capable of maintaining infections of two of the parasites found in trout. *Ptychogonimus fontanus* is a strongly suckered trematode found in perch and speckled trout. The only acanthocephalid found was present in both suckers and the speckled trout.

Host Parasite List

Catostomus commersonii
- *Glaridacris intermedius* sp. nov.
- *Neoechinorhynchus cylindratus*
- *Rhabdochona laurentiana* sp. nov.

Cristivomer naymaycush
- *Eubothrium* (larval)
- Ichthyotaeniidae (larval)

Perca flavescens
- *Crepidostomum isostomum*
- *Ptychogonimus fontanus*

Salvelinus fontinalis
- *Crepidostomum cooperi*
- *Ptychogonimus fontanus*
- *Eubothrium salvelini*
- *Eubothrium* (larval)
- Ichthyotaeniidae (larval)
- *Neoechinorhynchus cylindratus*
- *Cystidicloides harwoodi*
- *Raphidascaris alius* sp. nov.

Acknowledgments

The assistance of the Director and staff of the Institute of Parasitology, the co-operation of the North Lake Fish and Game Club, and the privileges granted by the Department of Mines and Fisheries of the Province of Quebec were important to this work and are gratefully acknowledged.

Names of fish appearing here are those used by Hubbs (9).

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PARACEONOGONIMUS KATSURADI SP. NOV. (TREMATODA : STRIGEIDA) FROM *LOPHODYTES CUCULLATUS* IN QUEBEC[1]

BY L. L. LYSTER[2]

Abstract

Paraceonogonimus katsuradi sp. nov. is described from *Lophodytes cucullatus*. The sub-subfamily Prohemistomini Dubois, 1938, and the genus *Paraceonogonimus* Katsurada, 1914, must be slightly modified to accommodate it.

In the course of a search for heterophyid infections in fish-eating birds in the Lake Commandant area in the Province of Quebec, trematodes were found in the intestine of *Lophodytes cucullatus*, the hooded merganser, which upon casual examination appeared to belong to *Apophallus*. It was only when specimens were stained and mounted and the holdfast organ recognized that it became apparent that these were members of the super-super-family Strigeida Poche, 1935. The lack of marked fore- and hind-body differentiation and the pyriform shape established the relationship to the super-family Cyathocotylides Dubois, 1936, and the family Cyathocotylidae Poche, 1925. The nature of the holdfast organ, the testes, the vitellaria, and body shape were those found in the super-subfamily Prohemistominae Lutz, 1935. Though the vitellaria were not entirely limited to the post-acetabular region as in the sub-subfamily Prohemistomini, the material seemed of such obvious relationship that it was assigned to this group. The presence of an acetabulum, the lack of a specialized terminal structure, and the absence of a marked ventral pit then identify this parasite as a member of the genus *Paraceonogonimus* Katsurada, 1914. The definition of the genus describes it as a Prohemistomini in which the body is ovoid. In order that the present species may be included it is proposed that this be modified to "ovoid or pyriform."

The genus was established for the type species *P. ovatus* (3). Ransom (4), misinterpreting the nature of the holdfast organ, considered it to be a Heterophyid related to *Cryptocotyle*. Ciurea (1) somewhat tentatively had suppressed the genus earlier in favour of the strigeid genus *Prohemistomum* Odhner, 1913. Szidat (5) later showed that the genus was a valid member of the Strigeida, and Dubois (2), in his monograph, treats it as a member of the sub-subfamily Prohemistomini, placing it midway between the genera *Linstowiella* and *Prohemistomum*.

[1] *Manuscript received July 22, 1939.*
Contribution from the Institute of Parasitology, Macdonald College, Que., with financial assistance from the National Research Council of Canada.
[2] *Graduate assistant.*

The sub-subfamily is defined as "Prohemistominae: vitelline follicles situated behind the acetabulum, placed near the middle of the body length, and disposed in a coronet around the holdfast organ, which is relatively well developed and hollowed by a cavity." In order that the material from the merganser may be accommodated, this definition must be slightly modified to include a species in which the vitelline follicles extend slightly anterior of the acetabulum, but are still limited to the posterior half of the body. The description of this detail would then be "Vitelline follicles situated behind the acetabulum or extending only slightly anterior to it."

The present material consists of eight specimens, of which five are egg-bearing. They are attenuate pyriform in shape, widest in the region of the holdfast organ and averaging 0.56 by 0.15 mm. in greatest measurement, with

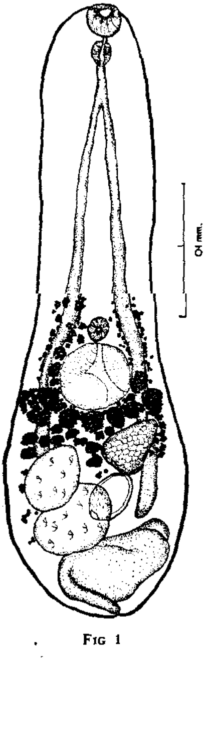

FIG 1

a maximum size of 0.63 by 0.168 mm. No cuticular spines were noted, but the cuticle was pitted as if spines might have been present before preservation. The organs are all situated in the posterior part of the body, and for this reason the post-acetabular portion is deeper than the fore part of the body, but though it is slightly hollowed ventrally there is no marked differentiation of this body region. The conspicuous but relatively weak holdfast organ, in the midline in the third quarter of the body, is 0.05 to 0.09 mm. in diameter. Anterior to it and from one-half to one-third its diameter is the acetabulum. The terminal oral sucker is 0.05 to 0.049 mm., slightly larger than the acetabulum, and is followed by a short pre-pharynx and a longitudinally ovoid pharynx about 0.05 by 0.034 mm. The oesophagus is two to four times the length of the oral sucker and opens into the crura far anterior of the acetabulum. The crura reach posteriorly to the posterior testis. The small vitellarian follicles surround the holdfast organ and are continued anterior to the acetabulum with a distinct series of follicles outlining the crura in this region; dorsal follicles are continued to the anterior region of the rear testis. The genital pore is terminal; a large and variously convoluted cirrus sac lies in the posterior extremity encroaching dorsally and laterally into the posterior testicular area, but not extending anterior of the posterior testis. The two testes are somewhat ovoid, oblique and contiguous, lying slightly to the left of the body and separated only by the cirrus sac from the posterior end. The posterior member, 0.075 by 0.08 to 0.09 mm., is larger than the other, 0.06 to 0.07 by 0.4 to 0.45 mm. The ovary, 0.05 to 0.07 mm. in greatest measurement, is roughly triangular, tending to be intermediate between the testes but on the opposite side of the body. The intra-uterine eggs, 84 by 70 μ, are always found singly.

This form differs from *P. ovatus* in several respects. It is pyriform in shape rather than ovoid, the vitellarian field is more extensive anteriorly but more limited posteriorly, and the cirrus sac is unlike that of *P. ovatus* in extent and organization. These differences serve to differentiate it from *P. ovatus*, and the material has therefore been treated as a previously undescribed species for which the name *Paraceonogonimus katsuradi* sp. nov., is proposed.

Specific Diagnosis

Paraceonogonimus: body pyriform, pre-pharynx present, oesophagus short; vitellaria surround holdfast organ dorsal and lateral to acetabulum and crura in this area, and extend dorsally to posterior testis; cirrus sac large but limited to area posterior of anterior margin of rear testis; testes oblique, ovary medial but on side of body opposite to testes.

Host: *Lophodytes cucullatus* (Hooded merganser).

Location: intestine.

Locality: Cameron's Bay, Lake Commandant, Argenteuil Co., Que..

Acknowledgments

The co-operation of the Director and staff of the Institute of Parasitology and the interest of Mr. Frank Dale, who took the mergansers, are gratefully acknowledged.

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INVESTIGATIONS ON TRICHINOSIS IN CANADA

III. ON THE INCIDENCE OF TRICHINOSIS IN GARBAGE-FED HOGS[1]

By Thomas W. M. Cameron[2]

Abstract

An examination of 995 garbage-fed hogs from Quebec, Ontario, and Manitoba, showed that only two harboured trichina larvae.

During the seasons 1937 and 1938, 2000 specimens of pork (1, 2) were examined at this Institute for the presence of trichinae. These specimens were taken at various abattoirs from Manitoba and Eastern Canada and were from *unselected* specimens. They were examined both by compression and by digestion of 10-gm. samples. They showed a general incidence of infection of 0.75%.

Recent research in the United States and elsewhere has suggested that meat-garbage fed to hogs is the main source of infection. In Canada, the Dominion Department of Agriculture requires all such garbage feeders to be licensed (and consequently to be under inspection); it further requires that all such garbage be cooked before feeding. There is no such general regulation in the United States, however. If the comparatively high incidence of trichinosis in garbage-fed hogs in that country (2) is due to this cause, it follows that, if the Department regulations in Canada are properly carried out, there should be little or no trichinosis in garbage-fed hogs in this country.

During the summer of 1939, through the co-operation of the Veterinary Director General, various abattoirs were requested to send to this Institute samples of diaphragm of hogs known to originate from hog breeders licensed to feed garbage. In this way 955 specimens were received. Apart from this, they were quite unselected. Specimens were received from Quebec, Ontario, and Manitoba only. The specimens were examined by techniques identical with those used in previous years and by the same technician who examined them in 1938. The distribution of samples is as follows.

Province	No. of samples	No. positive	Incidence
Quebec	225	2	0.88%
Ontario	283	0	0
Manitoba	487	0	0
	995	2	0.20%

[1] *Manuscript received October 7, 1939.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Professor, McGill University; and Director, Institute of Parasitology, Macdonald College, Que., Canada.*

Only two specimens contained trichina larvae. The first of these was one of a lot of 17 hogs, the second one of a lot of 10 hogs. Both lots originated in close proximity to the city of Montreal.

In the previous two years' investigation, 2,000 hogs were examined. These were unselected and consequently may have contained both garbage- and non-garbage-fed hogs. While not strictly comparable with this year's examination, the cumulative figures for the three years are as follows:

Province	No. of samples	No. positive	Incidence
Manitoba	1,253	5	0.39%
Ontario	834	3	0.36%
Quebec	725	8	1.10%
Maritime Prov.	157	1	0.65%
Saskatchewan	7	—	—
Unknown	19	—	—
Total	2,995	17	0.57%

The total of 2,995 hogs, of which at least a third are known to be fed on garbage, shows an incidence of 0.57%. The known garbage-fed hogs alone show an incidence of 0.20%.

It would appear legitimate to draw the following tentative conclusions from this survey.

1. The incidence of trichinosis in garbage-fed hogs in the United States is estimated at about 5%. The much lower figure found in this survey suggests that the cooking of garbage is a reasonably sound method of preventing the condition, even although an occasional case occurs. It is probable that such a case is due to insufficient cooking, although an extraneous source of infection cannot be excluded.

2. The general incidence of porcine trichinosis in Eastern Canada appears to be considerably lower than in the United States.

3. The comparatively high figures for the Province of Quebec are probably misleading. Two cases have been definitely traced to garbage-fed hogs. All the remaining six cases (reported in 1937) were recovered from one lot of seven samples received from a single centre and it is not unreasonable to assume that they represent a single infection; this infection is possibly traceable to garbage-fed hogs also although there is no information available.

4. The figures for the Maritime Provinces are based on too few samples to be reliable. It is more probable that a figure close to 0.4% (as shown by Ontario and Manitoba) is a more representative one. Much larger numbers are necessary, however, before this can be accepted. It is probable that even this low figure could be further reduced by a more strict application of the regulations regarding garbage cooking.

Acknowledgments

The examinations were made by Mr. Angus MacMillan, and the author has to thank Dr. A. E. Cameron, Veterinary Director General of Canada, for his co-operation in this survey, and Drs. G. A. Rose, Ed. Dufresne, Ed. Grandmaison, H. H. Anderson, R. D. Boast, and I. W. Purdy, for the actual collection of the specimens. These Inspectors' assistance was invaluable and all specimens received were in excellent condition.

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REPTANT DECAPOD CRUSTACEA OF THE WEST COASTS OF VANCOUVER AND QUEEN CHARLOTTE ISLANDS, BRITISH COLUMBIA[1]

By Josephine F. L. Hart[2]

Abstract

The range, size, local distribution, and notes on 44 species of reptant decapod crustaceans taken in British Columbia coastal waters are presented. A summary of records from the literature has been compiled, and the distribution of the species as a result of the peculiar configuration of the coast line is illustrated from these data.

The present paper records the range, size, local distribution, and notes on 44 species of reptant decapod crustaceans taken in the waters off the coast of British Columbia by collectors from the Pacific Biological Station. With these have been combined the records of the same species presented in the literature. Since the coast of British Columbia is probably unique in that it includes wide stretches of coast exposed to the open Pacific as well as equally large areas bordering sheltered inland passages, a summary of the records from the literature for the reptant decapods has been compiled from which an attempt is made to illustrate the difference in the distribution of the species as a result of the peculiar configuration of the coast line.

The material available consists of some 750 specimens, representing 44 species of reptant decapod Crustacea. The larger part of the collection was made in the summer of 1934 by Mr. E. G. Hart, while he was serving as Biologist for the Pacific Biological Station on the C.G.S. "Wm. J. Stewart" on the west coast of Vancouver Island. The remainder was obtained by Dr. C. McLean Fraser in 1935 when he was acting in a similar capacity on the west coasts of the Queen Charlotte Islands. The areas represented by the collections comprise the middle part of the west coast of Vancouver Island and the southern two-thirds of the west coasts of the Queen Charlotte Islands.

A few dredge hauls and shore collections in these areas, as well as along the remainder of the west coasts of Vancouver and Queen Charlotte Islands, were made by the United States Fisheries Steamer "Albatross" from 1888 to 1891 (1, 12–15). Collections were also made from the Queen Charlotte Islands by Dr. G. M. Dawson (Smith, 17), from Clayoquot, west coast of Vancouver Island, by Newcombe (10), from Ucluelet, Barkley Sound, west coast of Vancouver Island, by Macoun (Rathbun, 13–15), and from Clayoquot Sound by Spencer (18). A few records of species are also to be found in the lists of acquisitions of Crustacea in the Annual Reports of the British Columbia Provincial Museum (3). Only one-half the number of species in the present collection has been listed in these papers as occurring in localities on the west

[1] *Manuscript received October 9, 1939.*
Contribution from the Pacific Biological Station, Nanaimo, B.C.
[2] *Volunteer Investigator, Pacific Biological Station.*

coasts of Vancouver and Queen Charlotte Islands. The distribution records for the east coast of Vancouver Island, Victoria, and Friday Harbour, Washington, and northern British Columbia, are from papers listed in the references, with the exception of a few species that have been collected in the vicinity of Departure Bay by the author, but that have not been previously recorded.

One difficulty encountered is that a number of species have been reported in distribution records simply as occurring in British Columbia, since many of the early workers did not give specific localities, designating their specimens only as from "Vancouver Island", "Gulf of Georgia", "Queen Charlotte Island shore", etc. In view of the area included and of the great ecological differences involved, this is not at all satisfactory. The author has therefore endeavoured to be more specific, particularly in the case of species whose northern limit lies in British Columbia. When species are found on both east and west coasts of Vancouver Island, both localities are given as the northern range. This may be somewhat cumbersome, but is of value to local workers, and may be simplified with future collecting, when the species will perhaps be found to encircle the Island. Another difficulty is that two species have been confused, or have been recorded as one. Thus the early records for species of the genera *Petrolisthes*, *Chionoecetes*, and the rare species of *Cancer* cannot be used.

As will be seen by reference to the charts and tables included in this paper, the area concerned on the west coast of Vancouver Island was divided into a number of somewhat arbitrary regions, each of which contains consecutively numbered collection stations. The Barkley Sound area was designated as area 2211; Clayoquot Sound as 2221, 2222, and 2223; Nootka Sound as 2231 and 2232; and Esperanza Inlet as 2241, 2242, and 2243. The west coast of the Queen Charlotte Islands was considered as a unit and numbered area 3500.

In the following list, 11 species, whose northern limit lies in British Columbia, have been found farther north than previously recorded. The limit of distribution of those species that extend north and south of British Columbia has been obtained mainly from Rathbun's papers. The author is indebted to the Smithsonian Institution for the records of specimens of *Pagurus hemphillii* (Benedict) and *Orthopagurus minimus* (Holmes) from British Columbia in the collections of the United States National Museum, Washington, D.C. The size given is the length, or the length and breadth, of the carapace. The limits are the sizes of specimens in the collection.

Upogebia pugettensis (Dana)

Range. Southeastern Alaska to San Quentin Bay, Lower California.

Size. ♀ 14–35 mm. ♂ 10–37 mm.

Local distribution.	Esperanza Inlet area,	2241–13,	2 ♀	2 ♂
	Queen Charlotte Islands,	3529,		2 ♂

TABLE I

DATA CONCERNING COLLECTION OF MATERIAL

Station No.	Latitude N	Longitude W	Date	Method of collection
2211- 1	48°39′	125°46′	May 8, 1934	T 70 m.
2221- 3	49°07′45″	126°31′30″	15,	T 130 m.
8	49°10′30″	126°13′30″	14,	T 56 m.
11	48°52′	126°11′	1.	T 101 m.
14	49°46′15″	125°57′	July 5,	T 77 m.
16	48°59′45″	126°07′45″	May 1,	T 73 m.
17	49°00′15″	126°07′15″	1,	T 73 m.
18	49°02′45″	126°02′30″	1,	T 60 m.
20	49°00′15″	125°44′30″	11,	T 18–21 m.
22	49°19′45″	126°21′45″	16,	T 50 m.
25	49°14′	126°20′	23,	T 64 m.
26	49°11′	126°11′	23,	T 45 m.
27	49°10′30″	126°10′15″	23,	T 50 m.
28	49°11′30″	126°39′	June 5,	T 118 m.
2222- 4	49°09′45″	126°00′	11,	S
6	49°15′15″	126°07′	12,	S
2223- 1	49°28′30″	126°23′45″	May 29,	S
3	49°22′15″	126°28′45″	June 1,	S
2231- 6	49°08′30″	126°49′	5,	T 170–180 m.
9	49°10′	126°43′	5,	T 155 m.
10	49°13′30″	126°43′	5,	T 113 m.
13	49°21′	126°43′30″	9,	T 55 m.
14	49°23′45″	126°34′15″	May 31,	S
15	49°23′45″	126°34′15″	June 2,	S
16	49°34′45″	126°40′15″	13,	S
17	49°36′45″	126°48′15″	14,	S
18–I	49°33′30″	126°38′45″	18,	B 89 m.
19	49°31′15″	126°42′45″	19,	T 73 m.
20	49°36′45″	126°49′	26,	S
28	49°36′	126°43′15″	July 9,	S
29	49°36′	126°43′15″	10,	S
30	49°37′15″	126°49′30″	11,	S
31	49°22′15″	126°54′	12,	T 75–90 m.
32	49°31′45″	126°34′45″	13,	S
33	49°22′30″	126°55′	17,	T 137 m.
34	49°30′	126°51′	17,	T 95 m.
2241- 8	49°39′15″	126°57′15″	18,	B 45 m.
9	49°37′15″	126°50′30″	June 16,	S
11	49°40′45″	126°53′30″	27,	S
12	49°51′	127°06′15″	28,	S
13	49°45′	126°59′	July 14,	S
15	49°38′45″	127°00′	19,	T 73 m.
2242- 2	49°51′15″	127°04′45″	June 25,	S
3	49°44′	126°57′	July 24,	S
4	49°44′	126°57′	25,	S
5	49°44′	126°57′	26,	S
6	49°48′	126°58′15″	27,	S
3507	West side Louscoone Bay, 1 mile from entrance		June 4, 1935	S
3513	Head of Big Inlet		6,	T
3515	Rennell Sound between Gospel Is. and south shore.		10,	T
3516	Northeast of Marble Is.		11,	T
3519	Tasu Hbr., north shore 2 miles from entrance		14,	T
3521	Near Rose Hbr., Houston-Stewart Channel		18,	S
3525	Rennell Sound		24,	T
3526	9¼ mi. south of Marble Is.		26,	B

TABLE I—*Concluded*

DATA CONCERNING COLLECTION OF MATERIAL—*Concluded*

Station No.	Latitude N	Longitude W	Date	Method of collection
3527	Near south shore, Tasu Hbr.		27,	T
3529	Canoe Passage, Skidegate Ch.		30,	S
3530	West side, eastern Skidegate Narrows		July 1,	S
3532	Skidegate Channel, near western Narrows		3,	S
3536	Flamingo Hbr.		13,	From kelp
3537	South shore, Houston-Stewart Channel		16,	S
3539	Half-tide rock, entrance Flamingo Hbr.		17,	S
3540	Rocks, entrance to Big Inlet		18,	S

T—trawl; S—shore; B—bottom.

Munida quadrispina Benedict

Range. Sitka, Alaska to Los Coronados, Lower California.

Size. ♀ 10–23 mm. ♂9.5–73 mm.

Local distribution.	Barkley Sound area,	2211–1,		1♂
	Nootka Sound area,	2231–6,	1♀	1♂
		2231–10,	1♀	7♂
	Queen Charlotte Islands,	3516,		1♂
		3525,	2♀	

Petrolisthes eriomerus Stimpson

Range. Flamingo Inlet, Queen Charlotte Islands, B.C., to Lower California.

Size. ♀ 6 × 6–12.5 × 12.5 mm. ♂7.5 × 6.5–14.5 × 14 mm.

Local distribution.	Clayoquot Sound area,	2221–25,	1♀	
		2223–3,	1♀	
	Nootka Sound area,	2231–30,	2♀	
	Esperanza Inlet area,	2241–13,		1♂
		2242–3,	1♀	
		2242–4,		3♂
	Queen Charlotte Islands,	3507,	4♀	1♂
		3539,		1♂

Remarks. This species has been previously recorded in British Columbia only from Victoria and Nanaimo, and thus its occurrence on the west coast of Vancouver Island and the southwest of the Queen Charlotte Islands constitutes a considerable extension of its range.

Petrolisthes cin.tipes (Randall)

Range. Rose Harbour, Houston-Stewart Channel, Queen Charlotte Islands, B.C., to Gulf of California.

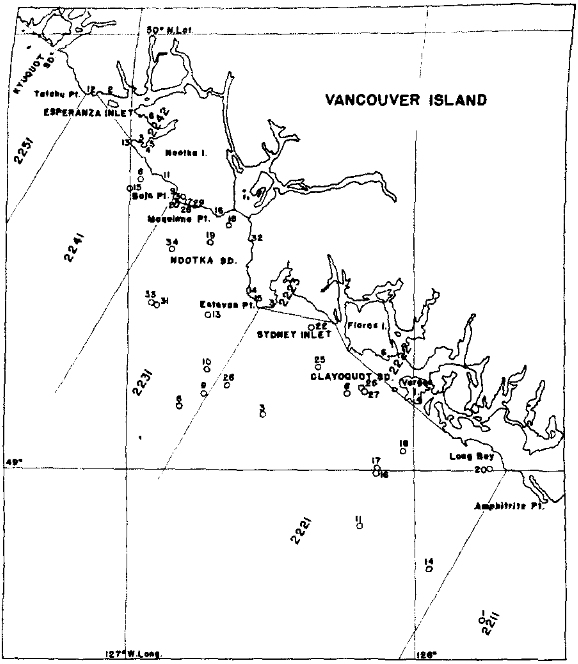

FIG. 1. *Showing locations of the following Stations: 2211-1; 2221-3, 8, 11, 14, 16 17, 18, 20, 22, 25, 26, 27, 28; 2222-4, 6; 2223-1, 3; 2231-6, 9, 10, 13, 14, 15, 16, 17, 18-1, 19, 20, 28, 29, 30, 31, 32, 33, 34; 2241-8, 9, 11, 12, 13, 15; 2242-2, 3, 4, 5, 6.*

Size. ♀10.5 × 10.5–24 × 24 mm. ♂10 × 9–17 × 16 mm.

Local distribution.	Clayoquot Sound area,	2222–6,	1 ♀	
	Nootka Sound area,	2231–14,	1 ♀	1 ♂
		2231–30,	1 ♀	
	Esperanza Inlet area,	2242–3,	1 ♀	
	Queen Charlotte Islands,	3507,		1 ♂
		3521,		3 ♂

Remarks. This species was recorded by the early naturalists from the east and west coasts of Vancouver Island. *P. eriomerus* Stimpson was not mentioned, and as Taylor (21) states that *P. cinctipes* was common at Nanaimo, where only *P. eriomerus* is now found, it is not possible to use

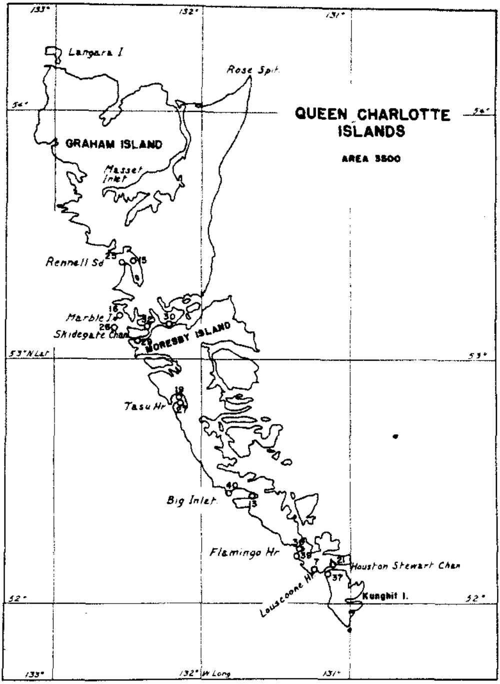

FIG. 2. *Showing locations of the following stations in the 3500 area: 07, 13, 15, 16, 19, 21, 25, 26-B, 27, 29, 30, 32, 36, 37, 39, 40.*

the early records. Since there has been no recent record of *P. cinctipes* from the east coast of Vancouver Island or from Puget Sound, it would appear that this species occurs in British Columbia only on the west coast of Vancouver Island and the Queen Charlotte Islands. Its northern known limit is extended considerably by this collection.

Pachycheles rudis Stimpson

Range. Kodiak, Alaska, to San Diego, California.

Size. ♀8 × 8–19.5 × 20.5 mm. ♂3.5 × 3.5–17 × 18 mm.

Local distribution.	Clayoquot Sound area,	2223–1,	1 ♀	1 ♂
	Nootka Sound area,	2231–16,	1 ♀	2 ♂
		2231–20,	2 ♀	
		2231–29,		1 ♂
		2231–30,	4 ♀	
	Esperanza Inlet area,	2242–3,	7 ♀	2 ♂
		2242–4,	2 ♀	6 ♂

Remarks. This species exhibits a great variety of colour combinations. Most are reddish in formalin, but some have a brown or purple tinge, and are mottled grey and fawn. Some looked bleached and the chelae are white.

Pachycheles pubescens Holmes

Range. Nuchatlitz Inlet, Esperanza Inlet, Vancouver Island, B.C., to Monterey Bay, California.

Size. ♂13 × 13.5 mm.

Local distribution. Esperanza Inlet area, 2242–4, 1 ♂

Remarks. This is the first British Columbia record of this species and is a considerable northern extension of its range. The carapace is brick red in formalin, except for the rostrum and posterior margin, which is grey mottled with brown. The granules on the chelae are white and the inner margin is reddish.

Pagurus alaskensis (Benedict)

Range. Siberia and Alaskan coast of Bering Sea to Oregon.

Size. ♀7.5–10 mm. ♂5.5–14 mm.

Local distribution.	Clayoquot Sound area,	2221–27,		1 ♂
	Nootka Sound area,	2231–6,	1 ♀	
		2231–13,	1 ♀	
		2231–19,		1 ♂
		2231–31,		3 ♂
	Esperanza Inlet area,	2241–15,	1 ♀	1 ♂

Pagurus aleuticus (Benedict)

Range. Bering Sea (Pribilof Islands) to Oregon.

Size. ♀7 mm. ♂7–10 mm.

Local distribution.	Nootka Sound area,	2231–9,		1 ♂
		2231–33,	1 ♀	1 ♂

Pagurus ochotensis (Brandt)

Range. Unalaska to San Diego, California.

Size. ♀4–11 mm. ♂5–14 mm.

Local distribution.	Barkley Sound area,	2211–1,		2 ♂
	Clayoquot Sound area,	2221–8,	1 ♀	1 ♂
		2221–20,	1 ♀	
		2221–26,		2 ♂

Nootka Sound area,	2231–19,	2 ♀	1 ♂
	2231–31,	2 ♀	1 ♂
Esperanza Inlet area,	2241–15,		1 ♂
Queen Charlotte Islands,	3519,	12 ♀	12 ♂
	3527,	1 ♀	1 ♂

Pagurus brandti (Benedict)

Range. Bering Sea (latitude of Pribilof Islands) southward to Oregon.

Size. ♀ 4 mm. ♂ 4 mm.

Local distribution. Queen Charlotte Islands, 3519, 1 ♀ 1 ♂

Pagurus dalli (Benedict)

Range. Bering Sea to Oregon.

Size. ♀ 3–4 mm. ♂ 6 mm.

Local distribution.	Clayoquot Sound area,	2221–8,		1 ♂
	Nootka Sound area,	2231–19,	5 ♀	

Pagurus confragosus (Benedict)

Range. Bering Sea (latitude of Pribilof Islands) to mouth of the Columbia River, Oregon.

Size. ♀ 10 mm.

Local distribution. Nootka Sound area, 2231–9, 1 ♀

Remarks. This species has not been recorded previously from British Columbia, although found in waters north and south of this region. The colour in formalin is light, mottled with pinkish-brown dorsally except on the chelae and the centre of the hard part of the carapace. There is a greater depth of colour on either side of the distal joints of the walking legs.

Pagurus kennerlyi (Stimpson)

Range. Aleutian Islands to Washington.

Size. ♀ 3.5–11 mm. ♂ 3.5–5 mm.

Local distribution.	Barkley Sound area,	2211–1,		1 ♂
	Clayoquot Sound area,	2221–8,	1 ♀	
		2221–17,		2 ♂
		2221–18,	1 ♀	

Pagurus beringanus (Benedict)

Range. Bering Sea (latitude of Nunivak) to Monterey, California.

Size. ♀ 5 mm. ♂ 3.5–5 mm.

Local distribution.	Clayoquot Sound area,	2221–18,	6 ♀	1 ♂
	Queen Charlotte Islands,	3519,		1 ♂
		3527,	1 ♀	

Pagurus setosus (Benedict)

Range. Kodiak, Alaska, to Santa Cruz Islands, California.

Size. ♀ 4–6 mm. ♂ 4–11 mm.

Local distribution.	Clayoquot Sound area,	2221–18,	1 ♀	
	Nootka Sound area,	2231–9,		2 ♂
	Queen Charlotte Islands,	3519,		1 ♂
		3525,	1 ♀	
		3530,		1 ♂

Pagurus hirsutiusculus (Dana)

Range. Aleutian Islands to San Diego, California. Japan. Siberia.

Size. ♀ 4–7 mm. ♂ 4.5–15 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		2 ♂
	Nootka Sound area,	2231–9,		1 ♂
		2231–28,	2 ♀	2 ♂
	Esperanza Inlet area,	2242–3,		1 ♂
	Queen Charlotte Islands,	3529,	2 ♀	
		3537,	1 ♀	

Pagurus granosimanus (Stimpson)

Range. Unalaska to Ensenada, Lower California.

Size. ♀ 6–8 mm. ♂ 7.5–9.5 mm.

Local distribution.	Clayoquot Sound area,	2222–6,	1 ♀	
	Nootka Sound area,	2231–15,		1 ♂
		2231–17,		1 ♂
		2231–20,	1 ♀	1 ♂
		2231–29,		1 ♂
	Queen Charlotte Islands,	3507,	1 ♀	1 ♂

Pagurus hemphillii (Benedict)

Range. Houston-Stewart Channel, Queen Charlotte Islands, B.C., to Monterey, California.

Size. ♀ 5–10 mm. ♂ 4–14 mm.

Local distribution.	Nootka Sound area,	2231–17,		2 ♂
	Esperanza Inlet area,	2241–12,		1 ♂
		2241–13,		2 ♂
		2242–3,	1 ♀	1 ♂
		2242–4,		1 ♂
	Queen Charlotte Islands,	3507,	1 ♀	1 ♂
		3537,		2 ♂

Remarks. These records are all north of any previously recorded specimens (those in the United States National Museum are from Barkley Sound). In formalin, the colour is dark red with white carapace dorsally, and the tips of the dactyls of all the legs and the propodi of the chelipeds are light coloured.

Paguristes turgidus (Stimpson)

Range. Rennell Sound, Queen Charlotte Islands, B.C., o San Diego, California.

Size. ♀ 4–13.5 mm. ♂ 4–22 mm.

Local distribution.	Barkley Sound area,	2211–1,		1 ♂
	Clayoquot Sound area,	2221–3,	1 ♀	2 ♂
		2221–14,	1 ♀	
	Nootka Sound area,	2231–6,	2 ♀	
		2231–9,	1 ♀	
		2231–10,	1 ♀	
		2231–13,	1 ♀	1 ♂
		2231–19,		1 ♂
		2231–31,	15 ♀	14 ♂
		2231–33,	5 ♀	15 ♂
		2231–34,	1 ♀	
	Esperanza Inlet area,	2241–15,	2 ♀	1 ♂
	Queen Charlotte Islands,	3515,	3 ♀	2 ♂
		3527,		1 ♂

Remarks. This species has not been recorded from the northern points previously.

Orthopagurus schmitti (Stevens)

Range. Esperanza Inlet, and Departure Bay, Vancouver Island, B.C., to Puget Sound, Washington.

Size. ♀ 3–6 mm.

Local distribution.	Barkley Sound area,	2211–1,	1 ♀
	Esperanza Inlet area,	2242–4,	6 ♀

Remarks. This is the first record of this species from outside waters, as it has been found previously only in Puget Sound and the Gulf of Georgia, B.C. It occupies empty *Serpulid* tubes.

Orthopagurus minimus (Holmes)

Range. Skidegate, Queen Charlotte Islands, B.C., to San Francisco, California.

Size. ♀ 3–5.5 mm. ♂ 4–5 mm.

Local distribution.	Queen Charlotte Islands,	3519,		1 ♂
		3527,	4 ♀	2 ♂

Remarks. Schmitt (16) gave the range as Queen Charlotte Sound instead of Queen Charlotte Islands. That this is an error has been verified for me by the United States National Museum. This species occupies empty *Dentalium* shells. The large chelae and the cephalothorax are deep red and the rest is straw-coloured in formalin.

Oedignathus inermis (Stimpson)

Range. Unalaska to Pacific Grove, California. Japan.

Size. ♀ 5 × 4–17 × 16 mm. ♂ 4 × 3.5–30 × 25 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		1 ♂
		2222–6,	1 ♀	
	Nootka Sound area,	2231–14,		2 ♂
		2231–15,		1 ♂
		2231–30,	1 ♀	
		2231–32,	1 ♀	
	Esperanza Inlet area,	2241–12,		1 ♂
		2242–3,	1 ♀	
		2242–4,		1 ♂
	Queen Charlotte Islands,	3539,	8 ♀	7 ♂
		3540,		1 ♂

Remarks. The coloration, in formalin, is usually red-brown with violet granules on the large cheliped. The claws of the walking legs are black, with deep yellow bands on the distal part of the propodi.

Hapalogaster mertensii Brandt

Range. Atka, Aleutians, eastward and southward to Puget Sound.

Size. ♀ 10 × 11–17 × 19 mm. ♂ 15 × 16–22 × 24 mm.

Local distribution. Queen Charlotte Islands, 3521, 2 ♀ 2 ♂

Remarks. The absence of this species from the west coast of Vancouver Island, is perhaps significant, but no definite statement can be made without more extensive distribution records.

Cryptolithodes sitchensis Brandt

Range. Sitka, Alaska, to Pacific Grove, California.

Size. ♂ 7 × 10–36 × 52 mm.

Local distribution.	Clayoquot Sound area,	2223–3,	1 ♂
	Nootka Sound area,	2231–17,	1 ♂
	Esperanza Inlet area,	2242–4,	2 ♂

Remarks. One specimen in formalin is brick-red dorsally, and white, tan and red, ventrally; another pearl-grey with a symmetrical pattern of dark red dorsally and with the dorsal side of the chelipeds and legs tan; another a dirty white; and the fourth, grey etched with black, and periopods brown dorsally.

Lopholithodes foraminatus (Stimpson)

Range. Clayoquot Sound and Nanoose Bay, Vancouver Island, to San Diego, California.

Size. ♀ 110 × 130–120 × 140 mm.

Local distribution. Clayoquot Sound area, 2221–3, 3 ♀

Remarks. The northern limit for this species is usually given as Victoria, although Taylor (21) reports seeing it at Nanaimo. There are specimens from Nanoose Bay, north of Nanaimo, in the Museum of the Pacific Biological Station, Nanaimo, B.C.

Oregonia gracilis Dana

Range. Bering Sea to Monterey, California. Japan.

Size. ♀11 × 7–35 × 19 mm. ♂7 × 5–40 × 20 mm.

Local distribution.	Barkley Sound area,	2211–1,	2 ♀	1 ♂
	Clayoquot Sound area,	2221–3,		1 ♂
		2221–8,	10 ♀	8 ♂
		2221–16,	34 ♀	31 ♂
		2221–17,	11 ♀	19 ♂
		2221–20,	3 ♀	9 ♂
		2221–22,	1 ♀	2 ♂
		2221–26,	7 ♀	3 ♂
		2221–27,		1 ♂
		2221–28,	1 ♀	2 ♂
		2223–1,	1 ♀	
	Nootka Sound area,	2231–13,	3 ♀	
		2231–19,	2 ♀	2 ♂
		2231–29,	1 ♀	1 ♂
		2231–31,	5 ♀	1 ♂
		2231–34,	4 ♀	7 ♂
	Esperanza Inlet area,	2241–8,		1 ♂
		2243–6,		1 ♂
	Queen Charlotte Islands,	3519,		1 ♂
		3527,		1 ♂

Pugettia producta Randall

Range. Houston-Stewart Channel, Queen Charlotte Islands, to Santa Rosalia Bay, Lower California.

Size. ♀13 × 9–50 × 42 mm. ♂25 × 21–40 × 28 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		1 ♂
	Nootka Sound area,	2231–28,	2 ♀	
		2231–32,		1 ♂
	Esperanza Inlet area,	2241–13,	1 ♀	
	Queen Charlotte Islands,	3521,		1 ♂

Remarks. This species has been previously recorded only from Vancouver Island southward.

Pugettia gracilis Dana

Range. Western extremity of the Aleutian Islands eastward and southward to Mendocino, California.

Size. ♀15 × 11–35 × 28 mm. ♂9 × 8–35 × 28 mm.

Local distribution.	Clayoquot Sound area,	2222–6,	1 ♀	
	Nootka Sound area,	2231–20,		2 ♂
		2231–28,		2 ♂
		2231–30,	1 ♀	
		2231–32,		1 ♂

	Esperanza Inlet area,	2241–11,		1♂
		2242–3,	1♀	1♂
		2242–4,	1♀	
	Queen Charlotte Islands,	3507,	5♀	1♂
		3521,	4♀	3♂
		3539,	3♀	5♂

Pugettia richii Dana

Range. Esperanza Inlet, Vancouver Island, B.C., to San Diego, California.

Size. ♀9 × 6–33 × 26.5 mm. ♂9.5 × 7–44 × 36 mm.

Local distribution.	Clayoquot Sound area,	2223–1,		1♂
		2223–3,		2♂
	Nootka Sound area,	2231–14,	1♀	1♂
		2231–17,		1♂
		2231–20,	1♀	1♂
		2231–28,	3♀	5♂
		2231–30,	3♀	
	Esperanza Inlet area,	2241–11,		1♂
		2241–12,	1♀	1♂
		2242–3,	6♀	3♂
		2242–4,	1♀	1♂
		2242–5,		1♂

Remarks. The northern limit of range of this species is extended by the present collection. There is considerable variation in colour, from red to brown, and the legs are usually banded with light and dark colour. A number of the specimens are covered with encrustations of bryozoans, hydroids, algae, etc.

Mimulus foliatus Stimpson

Range. Unalaska to Monterey Bay, California. Mexico.

Size. ♀11 × 11.5–23 × 24.5 mm. ♂11 × 10–35 × 39 mm.

Local distribution.	Clayoquot Sound area,	2223–1,		1♂
		2223–3,		1♂
	Nootka Sound area,	2231–20,		3♂
		2231–28,		1♂
		2231–30,	2♀	2♂
	Esperanza Inlet area,	2241–12,	1♀	1♂
		2242–3,	1♀	1♂
		2242–4,	2♀	3♂
		2242–5,		1♂
	Queen Charlotte Islands,	3507,		1♂
		3521,	1♀	

Remarks. There is considerable variation in the coloration, but the presence of a distinct V on the carapace seems to be fairly consistent. There are light coloured bands between the hepatic and lateral spines, which join

medially over the cardiac region. The predominant colour is red or red-brown, with a white V and red and white striped legs. The chelae are lighter in colour than the carapace.

Scyra acutifrons Dana

Range. Kodiak, Alaska, to San Diego, California.

Size. ♀13 × 9.5–36 × 26 mm. ♂10 × 8–46 × 35 mm.

Local distribution.	Nootka Sound area,	2231–16,	1 ♀	
		2231–20,	1 ♀	3 ♂
		2231–28,	3 ♀	1 ♂
		2231–29,	1 ♀	
		2231–30,	4 ♀	1 ♂
		2231–32,		1 ♂
	Esperanza Inlet area,	2241–12,	2 ♀	2 ♂
		2242–3,	4 ♀	
		2242–4,	3 ♀	7 ♂
		2242–5,	2 ♀	
	Queen Charlotte Islands,	3537,		1 ♂

Remarks. The carapace of the larger specimens is usually covered with an encrustation of sponges, tunicates, barnacles, etc. The tubercles are rose-coloured, and the legs banded with red and white.

Chorilia longipes Dana

Range. Shumagin Bank and Kodiak, Alaska, to San Diego, California. Japan.

Size. ♀8 × 4.5–34×20 mm. ♂7 × 4–21 × 11 mm.

Local distribution.	Clayoquot Sound area,	2221–16,	5 ♀	
		2221–17,	3 ♀	2 ♂
	Nootka Sound area,	2231–31,	1 ♀	
	Queen Charlotte Islands,	3526,		1 ♂
		3527,	2 ♀	1 ♂

Hyas lyratus Dana

Range. Bering Sea to Admiralty Inlet, Washington.

Size. ♀13 × 8.5 mm.

Local distribution. Nootka Sound area, 2231–31, 1 ♀

Cancer productus Randall

Range. Kodiak, Alaska, to Laguna Beach, California.

Size. ♀37 × 57–55 × 85 mm. ♂10 × 11–27 × 42 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		1 ♂
		2223–1,		1 ♂
		2223–3,		1 ♂
	Nootka Sound area,	2231–30,	1 ♀	
		2231–32,		1 ♂

	Esperanza Inlet area,	2241–13,	1 ♀	
		2242–3,		1 ♂
		2242–4,	1 ♀	

Remarks. The usual variety of colour in the small specimens is found: pure white; bands of brown and white; stripes of red and white; brown striped. The larger specimens are a uniform red.

Cancer magister Dana

Range. Unalaska to Monterey Bay, California.

Size. ♀ 35 × 50 mm. megalopae.

Local distribution.	Clayoquot Sound area,	2222–4,	1 ♀
	Nootka Sound area,	2231–28,	2 megalopae
	Esperanza Inlet area,	2241–9,	2 megalopae

Cancer branneri Rathbun

Range. Granite Cove, Port Althorp, Alaska, to Santa Catalina Island, California.

Size. ♀ 13 × 17–28 × 39 mm. ♂ 4.5 × 5.5–35 × 54 mm.

Local distribution.	Barkley Sound area,	2211–1,	2 ♀	
	Clayoquot Sound area,	2221–17,		1 ♂
		2221–20,	2 ♀	3 ♂
		2221–26,	12 ♀	7 ♂
		2221–27,	1 ♀	2 ♂
	Esperanza Inlet area,	2241–8,		2 ♂

Cancer oregonensis (Dana)

Range. Pribilof Island and Rat Island, Alaska, to Santa Barbara, California.

Size. ♀ 10 × 14–17 × 21.5 mm. ♂ 4.5 × 5–9.5 × 12 mm.

Local distribution.	Clayoquot Sound area,	2221–25,	1 ♀	
		2222–6,	1 ♀	
	Nootka Sound area,	2231–14,		1 ♂
	Esperanza Inlet area,	2241–13,	1 ♀	
		2242–3,		2 ♂
		2242–4,		3 young

Telmessus cheiragonus (Tilesius)

Range. Bering Sea to California.

Size. ♂ 7 × 7 mm.

Local distribution.	Queen Charlotte Islands,	3536,	1 ♂

Lophopanopeus bellus (Stimpson)

Range. Prince William Sound, Alaska, to Monterey, California.

Size. ♀ 8 × 11–15 × 22 mm. ♂ 13 × 19–20.5 × 30 mm.

Local distribution.	Nootka Sound area,	2231–20,	1 ♀	1 ♂
		2231–29,		2 ♂
		2231–30,	6 ♀	
	Esperanza Inlet area,	2241–12,		1 ♂
		2241–13,		1 ♂
		2242–3,	2 ♀	1 ♂
	Queen Charlotte Islands,	3507,		1 ♂

Pinnixa faba (Dana)

Range. Prince of Wales Island, Alaska, to Humboldt Bay, California.

Size. ♀ 5 × 9 mm. ♂ 6.5 × 11 mm.

Local distribution.	Nootka Sound area,	2231–14,		1 ♂
	Esperanza Inlet area,	2242–6,	1 ♀	

Remarks. The male is tan with a white mottled branchial region and the female is white, mottled with brown.

Pinnixa littoralis Holmes

Range. Sitka, Alaska, to San Diego, California.

Size. ♀ 11.5 × 18.5–16 × 20 mm.

Local distribution.	Esperanza Inlet area,	2242–3,	1 ♀
	Queen Charlotte Islands,	3532,	1 ♀

Remarks. The "liver" shows yellow and the ovary orange through the transparent carapace.

Pinnixa occidentalis Rathbun

Range. Unalaska to Magdalena Bay, Lower California.

Size. ♀ 2 × 4–2.5 × 5 mm. ♂ 2 × 5–3 × 6.5 mm.

Local distribution.	Queen Charlotte Islands,	3513,	12 ♀	9 ♂

Pinnixa schmitti Rathbun

Range. Port Levasheff, Alaska, to San Francisco Bay, California.

Size. ♀ 2.5 × 4.5 mm. ♂ 2.5 × 5 mm.

Local distribution.	Esperanza Inlet area,	2242–6,	1 ♀	1 ♂

Scleroplax granulata Rathbun

Range. Esperanza Inlet and Departure Bay, Vancouver Island, B.C., to Ensenada, Lower California.

Size. ♀ 4.5 × 6–5 × 6 mm.

Local distribution.	Esperanza Inlet,	2242–6,	3 ♀

Of the 44 species in the collection, two, *Pachycheles pubescens* Holmes and *Pagurus confragosus* (Benedict), have not been previously recorded from British Columbia. Five species found in the Queen Charlotte Islands collec-

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tion are not represented in the Vancouver Island collection, but as all of these range southward, it is probable that they occur here also. *Hemigrapsus nudus* (Dana) and *H. oregonensis* (Dana), the common shore crabs, do not occur in the west coast of Vancouver Island collection. Two explanations may be advanced: First, both species require the protection of gravel or loose rocks, which condition apparently is not commonly met with on that rugged, surf-washed shore; and second, at low tide the shore crabs are sufficiently high up on the beach to be entirely missed when collections are made near low water line. That these species do occur in this region there can be little doubt, as the larvae were present in the zooplankton taken from the same area. Moreover, Spencer (18) reports them abundant throughout Clayoquot Sound. Fraser did not collect these two species but identified them in the field at a number of Queen Charlotte Islands shore stations. He also identified, but did not collect, *Cancer magister* Dana, *C. productus* Randall, and *C. oregonensis* (Dana).

Table II is a compilation of the records of the 69 species of reptant Decapoda that have been found in British Columbia. The coast has been divided into eight regions: southern Vancouver Island (Victoria, B.C., and Friday Harbour, Washington); the east coast of Vancouver Island and the corresponding mainland coast; four areas on the west coast of Vancouver Island; the Queen Charlotte Islands; Queen Charlotte Sound, and northeast coasts. Records from published works are indicated by *p*, unpublished personal records by *H* and the present collection by *X*.

TABLE II

Occurrence of reptant Decapoda in British Columbia

	Gulf of Georgia	Victoria, B.C., and Friday Hbr., Washington	Barkley Sound	Clayoquot Sound	Nootka Sound	Esperanza Inlet	Queen Charlotte Islands	Queen Charlotte Sound and north
Munida quadrispina Benedict	*p*	*p*	*X*		*X*		*Xp*	*p*
Petrolisthes eriomerus Stimpson	*p*	*p*		*X*	*X*	*X*	*X*	
Petrolisthes cinctipes (Randall)				*Xp*	*X*	*X*	*X*	
Pachycheles rudis Stimpson		*p*	*p*	*Xp*	*X*	*X*		
Pachycheles pubescens Holmes						*X*		
Upogebia pugettensis (Dana)	*p*	*p*		*p*		*X*	*Xp*	
Callianassa californiensis Dana	*p*	*p*		*p*				
Callianassa gigas Dana		*p*						
Pagurus alaskensis (Benedict)	*p*	*p*		*X*	*X*	*X*		
Pagurus aleuticus (Benedict)	*p*	*p*			*X*			*p*
Pagurus ochotensis Brandt	*p*	*p*	*Xp*	*X*	*X*	*X*	*X*	
Pagurus brandti (Benedict)	*H*	*p*					*X*	*p*
Pagurus dalli (Benedict)	*p*	*p*		*X*	*X*			
Pagurus confragosus (Benedict)					*X*			
Pagurus cornutus (Benedict)								*p*
Pagurus gilli (Benedict)	*H*	*p*						
Pagurus beringanus (Benedict)	*p*	*p*		*X*			*X*	
Pagurus kennerlyi (Stimpson)	*p*	*p*	*X*	*X*				
Pagurus setosus (Benedict)	*p*	*p*		*X*	*X*		*X*	
Pagurus hirsutiusculus (Dana)	*p*	*p*		*X*	*X*	*X*	*X*	

TABLE II—*Concluded*

OCCURRENCE OF REPTANT DECAPODA IN BRITISH COLUMBIA—*Concluded*

	Gulf of Georgia	Victoria, B.C., and Friday Hbr., Washington	Barkley Sound	Clayoquot Sound	Nootka Sound	Esperanza Inlet	Queen Charlotte Islands	Queen Charlotte Sound and north
Pagurus granosimanus (Stimpson)	*p*	*p*		X	X		X	*p*
Pagurus hemphillii (Benedict)			*p*		X	X	X	
Pagurus tenuimanus (Dana)	H	*p*						*p*
Pagurus splendescens Owen	*p*	*p*						
Paguristes turgidus (Stimpson)	*p*	*p*	X	X	X		X	*p*
Parapagurus mertensii (Brandt)					*p*			
Orthopagurus minimus (Holmes)							X*p*	
Orthopagurus schmitti (Stevens)	*p*	*p*	X			X		
Hapalogaster mertensii Brandt	H	*p*					X	
Oedignathus inermis (Stimpson)				X	X	X	X*p*	
Acantholithodes hispidus (Stimpson)	*p*	*p*					*p*	
Placetron wosnessenskii Schalfeew		*p*					*p*	
Cryptolithodes sitchensis Brandt		*p*		X	X*p*	X	*p*	
Cryptolithodes typicus Brandt	*p*	*p*						*p*
Lopholithodes foraminatus (Stimpson)	*p*	*p*		X				
Lopholithodes mandtii Brandt	*p*	*p*	*p*					
Rhinolithodes wosnessenskii Brandt		*p*						*p*
Paralithodes rostrofalcatus McKay								*p*
Paralithodes camtschatica Tilesius							*p*	
Paralomis multispina (Benedict)							*p*	
Phyllolithodes papillosus Brandt	*p*	*p*						
Oregonia gracilis Dana	*p*	*p*	X	X*p*	X	X	X*p*	*p*
Pugettia producta Randall	*p*	*p*	*p*	X*p*	X	X	X	*p*
Pugettia gracilis Dana	*p*	*p*	*p*	X*p*	X	X	X*p*	*p*
Pugettia richii Dana		*p*	*p*	X*p*	X	X		
Mimulus foliatus Stimpson			*p*	X*p*	X	X	X	
Scyra acutifrons Dana	*p*	*p*	*p*		X	X	X*p*	*p*
Chorilia longipes Dana	*p*	*p*		X	X		X	*p*
Chionoecetes bairdi Rathbun	*p*							*p*
Chionoecetes angulatus Rathbun							*p*	
Hyas lyratus Dana	*p*	*p*		*p*	X			*p*
Telmessus cheiragonus (Tilesius)	*p*	*p*		*p*			X*p*	*p*
Cancer productus Randall	*p*	*p*	*p*	X*p*	X	X	*p*	*p*
Cancer branneri Rathbun	H		X*p*	X		X		
Cancer gracilis Dana	*p*	*p*		*p*				
Cancer magister Dana	*p*	*p*		X*p*	X	X	*p*	*p*
Cancer oregonensis (Dana)	*p*	*p*		X	X	X	*p*	*p*
Lophopanopeus bellus (Stimpson)	*p*	*p*		*p*	X	X .	X	
Pinnotheres pugettensis Holmes	*p*	*p*						
Pinnotheres taylori Rathbun	*p*		*p*					
Fabia subquadrata Dana	*p*	*p*					*p*	
Pinnixa faba (Dana)	*p*	*p*		*p*	X	X		
Pinnixa littoralis Holmes	*p*	*p*	*p*			X	X	
Pinnixa occidentalis Rathbun	*p*						X	*p*
Pinnixa schmitti Rathbun	H	*p*				X		
Pinnixa tubicola Holmes	*p*	*p*						*p*
Scleroplax granulata Rathbun	*p*	*p*				X		
Hemigrapsus nudus (Dana)	*p*	*p*		*p*				*p*
Hemigrapsus oregonensis (Dana)	*p*	*p*		*p*				*p*

***p* — previously recorded; H — in author's collection; X — in 1934 and 1935 collections.**

It is only the shore and shallow water forms that one would expect to be appreciably influenced by the different conditions found on the exposed west coast as compared with the sheltered waters of the Gulf of Georgia, which borders the east coast. Of these conditions the temperature of the water is probably the chief limiting factor, as was suggested for the difference in littoral distribution of *Pagurus beringanus* (6). The surface water temperatures of the west and south coasts of Vancouver Island are consistently lower than those of the Gulf of Georgia. As a result, at Victoria, this species may be collected at low tide, while to obtain specimens of a similar size at Nanaimo it is necessary to dredge in deeper water.

Of the 69 species recorded from British Columbia, 63 have been found in the waters surrounding Vancouver Island. More than half of these may be obtained at low tide. The remainder are normally taken only by dredging and thus are often rather poorly represented in collections. Two of these species, *Chionoecetes bairdi* Rathbun and *Pinnixa occidentalis* Rathbun, have been found only on the east coast of Vancouver Island, while four others, *Pagurus confragosus* (Benedict), *Parapagurus mertensii* (Brandt), *Placetron wosnessenskii* Schalfeew, and *Rhinolithodes wosnessenskii* Brandt have been found only on the south or west coasts. Since none of these species has been taken in large numbers, or often, the range will probably be extended by future collecting. Nine shore and shallow water forms have been found only on the south or west coasts. Of these, *Callianassa gigas* Dana and *Pachycheles pubescens* Holmes have been rarely taken, but *Petrolisthes cinctipes* (Randall), *Pachycheles rudis* Stimpson, *Pagurus hemphillii* (Benedict), *Oedignathus inermis* (Stimpson), *Cryptolithodes sitchensis* Brandt, *Pugettia richii* Dana, and *Mimulus foliatus* Stimpson seem to be fairly abundant on the west coast of Vancouver Island, and thus would appear to require the cold waters and rugged conditions associated with proximity to the open ocean.

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APOPHALLUS IMPERATOR SP. NOV., A HETEROPHYID ENCYSTED IN TROUT, WITH A CONTRIBUTION TO ITS LIFE HISTORY[1]

BY L. L. LYSTER[2]

Abstract

Apophallus imperator sp. nov., (Heterophyidae : Trematoda) is described and figured. It is a potential human parasite of which the metacercarial stage is encysted in *Salvelinus fontinalis* in the Province of Quebec. The other hosts are unknown; experimentally, it becomes adult in cats and pigeons. The significance of the genus *Apophallus* and related genera is reviewed and the systematics of *Apophallus* discussed. Organization of the gonotyl is the only feature accepted as uniformly distinctive of this genus and modifications in that structure are traced and recommended for their significance in specific diagnoses.

Introduction

In the course of a survey of parasitism in trout during the summer of 1938 an interesting condition was reported among Speckled Trout *(Salvelinu. fontinalis)* taken from parts of Lake St. Bernard in the Province of Quebec The fins and the skin under the scales in a specimen sent from this area were disfigured by numerous black spots. Microscopic examination showed tha this was caused by the encysted metacercaria of some trematode. To establish the identity of the parasite, part of the trout was given to a cat that hac never previously been fed fish. On post-mortem examination of the ca 12 days later, several minute flukes, easily recognizable as members of the family Heterophyidae, were recovered.

Recent studies have illustrated the economic interest of the Heterophyidae The lack of host specificity among many genera of the family makes it important to fur-bearing animals and wild life as well as to man. Human heterophyidiasis has been known for many years, and Africa, Garcia, and de Leon in the Philippines have demonstrated a potential high pathogenicity in human infections (2, 4). Not only is the intestinal mucosa parasitized but the eggs of the parasite may be carried by the blood stream to the heart and the central nervous system with serious effects. Cameron (8) recently recovered unidentified heterophyid eggs from a patient in a local hospital, the first recorded human case in North America.

Various marine and freshwater fish are known to be heterophyid vectors One of the carriers of *Metagonimus* in Japan is a salmonid fish, but no infection in North American species of these fish has been reported.

Trout carrying the metacercariae were easily obtained from the southern end of Lake Commandant, Argenteuil Co., Quebec. The condition has been

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known to exist there for several years and all experimental material came from there.

Description (Fig. 1)

Body linguiform, pyriform, or discoid, covered with spines in parallel rows, most numerous from oral sucker to intestinal bifurcation, very few posterior to testes and none at extreme anterior tip. Oral sucker subterminal, 0.063 by 0.056 mm. Pre-pharynx very short, usually entirely hidden behind the oral sucker. Pharynx longitudinally ovoid. Oesophagus long, weak, extending for one-half length of body, or somewhat less. Caeca larger than oesophagus, extending to well behind testes. Acetabulum relatively well developed and large, 0.077 by 0.070 mm., medial and posterior to intestinal furcation. Genital sinus opening anterior to ventral sucker; two gonotyls dorsal and anterior to acetabulum, 0.021 by 0.027 mm., narrowly separated. Seminal vesicle large structure dorsal and posterior to acetabulum in midline, curving ventrally and laterally, narrower terminal portion; dorsal and lateral portions sac-like and separated by constrictions. Ovary pyriform, 0.026 to 0.031 mm. by 0.056 to 0.077 mm., on left side of body nearer to ventral sucker than to testes. Seminal receptacle large, globular, anterior to ovary. Testes two, 0.057 to 0.063 mm. by 0.112 to 0.126 mm., triangular in shape, usually oblique but sometimes almost side by side, or almost tandem; right member anterior; posterior member removed from end by about its own diameter. Vasa efferentia continue in vas deferens to join seminal vesicle left of acetabulum. Uterus short, few turns, contains 5 to 20 large brown eggs, 0.014 to 0.019 mm. by 0.033 to 0.035 mm., reaches genital opening from left side of acetabulum. Common vitelline ducts rise at level of anterior testis. Vitellaria rise at level of gonotyl, extend to posterior end in a narrow limited band of scattered follicles, extending towards median field immediately behind testes and at level of common vitelline duct, but rarely meeting. Excretory bladder S-shaped with arms to pharyngeal area.

Variations in Morphology

All adult specimens were treated in the same way. The intestinal mucosa of the experimental animal was scraped into saline and sedimented with the addition of a little sodium bicarbonate. The living flukes were pipetted into cold fixative (glacial acetic formalin alcohol) and shaken. They were preserved in a mixture of 5% glycerine in 70% alcohol, to which had been added 5% formalin, then washed in distilled water and dilute alcohol and stained in acid alum carmine. In spite of this, there is a wide range of body shape (Figs. 4–7). Those from the pigeon are more uniformly pyriform, those from the cat usually ovoid, constricted in the region of the ventral sucker.

Two specimens show vitellaria extending anteriorly as far as the bifurcation of the oesophagus. In all others, the vitellaria are restricted to an area behind the gonotyl. In one of the two variant specimens the ventral sucker has been displaced antero-laterally and lies up the right side of the body near

the base of the oesophagus. In both, the testes are almost tandem and removed posteriorly. The status of these forms is difficult to determine. Important features have been obscured in mounting; one might well be *Apophallus venustus* but the other seems definitely a malformation of the present species.

Identification

This form, obviously a heterophyid, is also recognizable as a member of the genus *Apophallus sensu latu*. This group is poorly understood. In order that identification may be clear some discussion of the related genera that include *Apophallus* is necessary here. It is limited as far as possible to *Apophallus* and the genera that have been confused with it.

History

The genus *Apophallus* was created in 1909 by Luhe (18) with *Distomum muhlingi* Jagerskiold, 1899, from *Larus ridibundus* as type species; it was incorrectly described as *Distomum lingua* by Creplin in 1898.

In 1919 Skrjabin and Lindtrop (23) erected the genus *Rossicotrema* with *R. donicum* as type species, from the cat and dog, distinguishing it from *Apophallus* because of the condition of the vitellaria and testes.

Without reference to that genus, Ransom in America created the genus *Cotylophallus* in 1920 (22) based on the arrangement of the vitellaria; it contained two species, *C. venustus* from the cat, dog, and wolf, and *C. similis* from *Phoca vitulina*, those also being distinguished by vitellaria arrangement. In the same paper he described *A. brevis* from *Larus delawarensis*.

All subsequent authors have treated *Cotylophallus* as a synonym of *Rossicotrema*.

In 1924, Szidat (25) described *Apophallus major* from *Larus fuscus*, basing his new species only on absolute measurements.

In 1931, Price (20) described *A. crami* from *Larus californicus*, in which the vitellaria were limited to the post-ovarian region. A year later (21) he created *A. zalophi* from *Zalophus californicus*, differing widely from the type in organization of the vitellaria, development of the pre-pharynx and other details. The species *A. americanus* was described on two immature specimens from the stomach of fish in 1932 by Van Cleave and Mueller (27). The specimens were apparently ectopic forms and the specific identification is based on features now known to be variable. The species *americanus* may well be valid, but in reporting it the authors imply that they are creating it largely to avoid confusion until further material is available.

In 1935, Africa (3) described the species *eccentricus* which he referred to *Apophallus*, though it differed in several details usually considered supergeneric and mainly in the position of the gonotyl.

Thus, two closely related genera, *Apophallus* and *Rossicotrema*, containing nine species between them, have been introduced into the literature. These Ciurea (9) placed together in his sub-family Apophallinae, because of common

characters of the acetabulo-genital complex (notably that the genital sinus opens anterior to the acetabulum).

Witenberg (28), reviewing the family, placed *Apophallus* and *Rossicotrema* together with *Cryptocotyle* and *Tocotrema* (in which the genital sinus opens posterior to the acetabulum) in the new tribe *Cryptocotylea*, and in the sub-family Heterophyinae which included the sub-families Metagoniminae, Apophallinae, and Cryptocylinae as understood by Ciurea. He considered the arrangement of the acetabulo-genital complex of sub-generic importance and differentiated the tribe solely on the anterior extent of the vitellaria. This division would remove *A. crami*, in spite of its very obvious relationships, to his tribe Heterophyea, where there is no genus to accommodate it. Witenberg refused to accept comparison of vitellarian fields for separating species. He reduced *R. donicum*, *R.* (*-Cotylophallus*) *similis* and *R.* (*-Cotylophallus*) *venustum* to synonymity, and transferred *A. brevis* to this genus, making it a synonym of *R. donicum*. He discounted the specific importance of size and made *A. major* a synonym of *A. muhlingi*.

In 1930 (29), in amending his previous monograph in several details, he made the genus *Rossicotrema* a synonym of *Tocotrema* Looss, 1899. He considered the condition and position of the acetabulum and position and number of gonotyls of specific interest only. Stunkard (24), in describing the life history of the type species, *Tocotrema lingua*, did much to establish its congenerity with *Cryptocotyle* Luhe, 1899, a relationship that had previously been accepted by all workers with two exceptions (Ciurea (10) and Linton (16)).

Price (20), in 1931, considered that the acetabulo-genital complex furnished generic characters. Thus, he regarded *Rossicotrema* as distinct from *Tocotrema* (and, therefore, from *Cryptocotyle*) but indistinguishable from *Apophallus*. In the last genus he recognized *muhlingi*, *crami*, *donicus* (= *venustus* and *similis*), and *brevis*, as valid species, all separable on features of the vitellaria. Ciurea (10) did not agree that *Apophallus* and *Rossicotrema* could be synonyms, because of variations in body shape, arrangement of testes, and development of metacercariae. He concurred, however, in the differentiation of *Tocotrema* and *Rossicotrema*, which he assigned to the sub-families Cryptocotylinae and Apophallinae respectively.

The sub-family Cryptocotylinae had been created by Luhe (18), 1909, to contain the genera Cryptocotyle and *Scaphanocephalus*. To these Ciurea added the genera *Tocotrema* and *Ciureana*. The sub-family is characterized mainly by the reduced condition of the acetabulum and genital sucker and the position of the genital opening posterior to the acetabulum.

The sub-family Apophallinae, created by Ciurea (9), is based on the extent of development of the genital sucker and acetabulum and the position of the genital opening, anterior to the acetabulum. He has placed the genera *Apophallus*, *Rossicotrema*, *Euryhelmis*, and *Pricetrema* in it. The genus *Pricetrema* was erected at this time by Ciurea to accommodate *A. zalophi* Price, 1931. It is separated because of the length of the oesophagus, the situation of the testes, and the course of the seminal vesicle and uterus.

Poche (19) created the genus *Euryhelmis* to include *Distomum squamula* Rudolphi, 1819, but incompletely described it. Recently Baer (6), 1931, has made a more definite contribution to our knowledge of the morphology of this form. He points out the unique organization of the acetabulo-genital complex. The genital sucker, situated anterior to the acetabulum, consists of a *single* papillae-like muscular structure attached anteriorly. Very recently a description and life history of a new species, *Euryhelmis monorchis*, has been published by Ameel (5). In spite of its single testis, he placed it in the sub-family Heterophyinae *sensu* Witenberg, but his observations refute rather more than support the inclusion of this genus with *Apophallus* in the restricted sub-family Apophallinae *sensu* Ciurea.

Cameron (7) in a discussion on the family in 1936 accepted the synonymity of *Apophallus* and *Rossicotrema* but discounted the importance of body shape and size as a diagnostic character. He accorded only limited value to testicular arrangement for separating species but he acknowledged the importance of the vitellarian field. By this criterion he was able to distinguish *A. venustus* from *A. donicus*, *muhlingi*, and *brevis*, but not from *A. similis*, which he concluded to be a synonym.

It is apparent from this brief review that a great deal of confusion has resulted in these genera because of lack of agreement as to what should constitute generic and specific features.

The Genus

The genus *Apophallus* has previously been recorded under various synonyms. Price and Cameron consider *Rossicotrema* synonymous with it. *Rossicotrema* has been accepted as synonymous with *Cotylophallus*, and Witenberg considered it as a synonym of *Cryptocotyle* by various workers. *Pricetrema* was created by Ciurea to accommodate a species originally placed in *Apophallus*.

Some convention must be accepted to facilitate classification of this group. The obvious variation in the associated genera under discussion is in the structure of the acetabulo-genital complex. This feature has been variously considered of specific, generic, and super-generic significance.

Should the specific valuation be accepted, as recommended by Witenberg (28), a confusing group of synonymous designations results. No logical series of generic and specific characteristics can be traced. The variation in the complex seems to divide this part of the family into two distinct groups as represented by the genera *Cryptocotyle* and *Apophallus*. In the first a *single* gonotyl guards the genital opening *posterior* to the acetabulum. In the second, a *pair* of gonotyls guards the genital opening *anterior* to the acetabulum.

At the moment it is difficult to evaluate fully the true significance of this division, whether generic or super-generic. Two complementary factors must be considered:

1. The position of the genital opening.
2. The number of gonotyls.

The species *A. eccentricus* is unique because of the lateral opening of its genital pore. If valid, this species may represent a series of undiscovered forms which would be intermediate between the two genera and make *Apophallus* a synonym of *Cryptocotyle*. However, re-study will probably illustrate its closer relationship to *Stictodora* than to *Apophallus*.

A single gonotyl seems a constant feature among the genera in which the genital opening is posterior. With two exceptions all species with the anterior genital opening have two gonotyls. These exceptions, *Euryhelmis squamula* and *E. monorchis*, are of poorly understood phylogeny. They may represent a parallel organization which would place *Euryhelmis* in the same sub-family as *Apophallus* (as Ciurea (10) has done); they may be a continuation of the *Apophallus* type of development and therefore be congeneric with it; or, as available evidence suggests, they may have developed distinctly from *Apophallus* and thus bear no relationship to this genus. Present evidence, then, does not support super-generic or specific evaluations of these major features of differentiation, although in theory either may be correct. Following Price (20) and Cameron (7), generic rank of this character seems most practicable.

Genus *Cryptocotyle*

Reduced acetabulum, single gonotyl, posterior genital opening.

This genus is of no further interest in the present discussion.

Genus *Apophallus* (= *Pricetrema*, *Cotylophallus*, and *Rossicotrema* (not synonymous with *Tocotrema*).

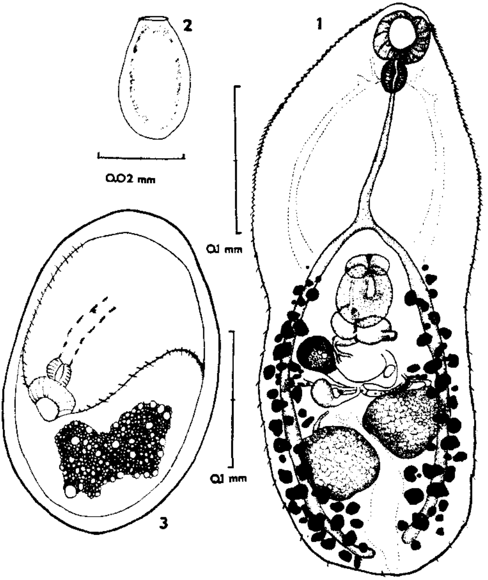
More distinct acetabulum, paired gonotyls, anterior genital opening.

This genus is essentially the same as Ciurea's sub-family Apophallinae, with the exclusion of the genus *Euryhelmis*. The present species is assigned to it.

The Species

Less clearly indicated are the specific characters; Ciurea, Africa, and Cameron and the present paper have proved the inconstancy of shape and size. The relative position of testes is variable within some species, more constant in others, but never suitable as a specific feature. Many authors have accepted the extent and arrangement of the vitellaria for diagnosing species. By this feature *A. venustus*, in which the vitellaria reach the crural furcation, is separable from *A. donicus* and *A. brevis*, in which the follicles do not exceed the gonotyls. In studying *A. donicus* (from the collection of Dr. Ciurea) and *A. brevis* (from the collection of Dr. Cameron) I find that the anterior limit of the vitellaria is constant but does not provide differences for complete differentiation of these species. From the same material it is seen that there is considerable variation in the medial follicles within each species. In *A. brevis*, the vitellaria meet behind the testes, but in front of these organs they are usually limited to the lateral fields. In a few specimens, however, they almost meet anterior to the ventral sucker. In *A. donicus*, the same condition may be noted, but in reverse order. The vitellaria usually meet in the midline anterior to the ventral sucker, but in a few specimens they

are limited to the lateral fields in this area. (Cameron's statement that the vitellaria did *not* reach "the ventral sucker" in these forms is probably a *lapsus*, intended for "the intestinal bifurcation".) These variations demonstrate the fact that the arrangement of the vitellaria is not acceptable for identifying species when considered alone. Further less variable characters must be found.

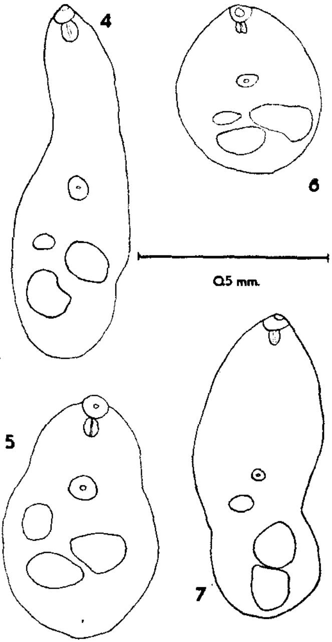


FIGS. 1–3. *Apophallus imperator sp. nov.* FIG. 1. *Adult from experimental cat.* FIG. 2. *Eggs from faeces of same host.* FIG. 3. *Encysted metacercaria from trout.*

Absolute measurements have not been accepted, but comparative measurements and morphology, especially of the more heavily muscled structures, should be valuable. The more strongly organized and least variable structures are the seminal vesicle, the oral sucker, and the acetabulo-genital complex. Of these, modification in the seminal vesicle is seen upon study to be a superspecific factor, and the oral sucker is important when considered in comparison with the ventral sucker, but does not afford a complete range of variations. Of the three, the acetabulo-genital complex seems likely to be the most satisfactory structure upon which to base criteria. The genital

sucker is most subject to the evolutionary changes that characterize the family to the extent that it has been assigned a special term "gonotyl." The genus, too, is identified by the condition of the remnant of this organ. Each species then might show a distinct development of the important structure.

The acetabulo-genital complex was studied in the three species of the genus *Apophallus* that have been confused in North American reports, *donicus*, *venustus*, and *brevis*, and in the present form and found to be separable. In *A. venustus* the ventral sucker is strongest; anterior to it is the genital structure, weakly muscled but illustrating distinctly its affinity to the true genital sucker of other trematodes, and lacking the extensive papillae-like construction of the organ as found in the other species of the genus (Fig. 8).



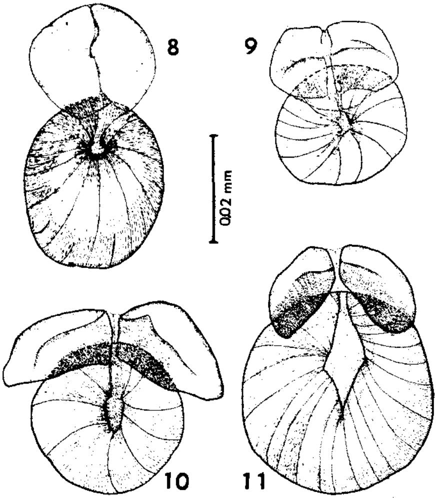
FIGS. 4 – 7. *Apophallus imperator sp. nov.* FIGS. 4 AND 5. *Outlines of adults from pigeon.* FIGS. 6 AND 7. *Outlines of adults from cat.*

In the remaining species the ventral sucker is less strongly muscled, and the genital structure is less weak. In *A. donicus* the papilla-like development is also somewhat limited, but not to the same extent as in *A. venustus* (Fig. 9). In *A. brevis* and the present form the structure consists of two distinct and strong bodies with little apparent relation to a primitive sucker. In the former species these gonotyls are large and extend laterally well beyond the acetabulum (Fig. 10), while in the latter they are roughly unilateral with this organ (Fig. 11).

Thus, two types of variable development are found in the remnant of the genital sucker in these species:

1. Papillae-like, far removed from progenital type—
   brevis—gonotyls large, extend laterally beyond margin of acetabulum.
   present species—gonotyls not so large, do not extend past lateral margins.
2. Not papillae-like, showing affinity to true genital sucker—
   venustus—gonotyls compound globular structures.
   donicus—gonotyls closely associated but more distinct than in *venustus*.

The remaining species are already easily distinguishable, with the exception of *muhlingi*.



FIGS. 8 – 11. *Acetabulum and gonotyl of Apophallus spp.* FIG. 8. *A. venustus.* FIG. 9. *A. donicus.* FIG. 10. *A. brevis.* FIG. 11. *A. imperator.*

A. muhlingi and the present form have some otherwise unique features in common. In each the vitellaria reach just to the level of the gonotyls and never extend medially except behind and between the testes. Similarly the acetabulum in each is usually slightly larger than the oral sucker. Though no specimens of *A. muhlingi* were available for comparison, Ciurea's careful discussion on it provides details for differentiation (10). The eggs in *A. muhlingi* are much broader than in the present form, though of approximately the same length. Of greater value is the nature of the gonotyls. He describes these organs as being symmetrical hemispheres with a diameter of 0.017 mm. His illustration shows a pair of such structures with the complete diameter parallel to the axis of the body. In the present form the gonotyls are 0.021 mm. in this measurement and 0.021 mm. transversely. Thus, the gonotyls of the two forms vary in size, shape, and direction of greatest measurement, though they are the most nearly alike of the species discussed.

Though it is closely related to *muhlingi* and *brevis*, the writer is thus able to distinguish this trout-carried member of the genus and considers it to represent a hitherto undescribed species, for which the designation *imperator* is proposed (from the locality in which it was discovered, Lake Commandant).

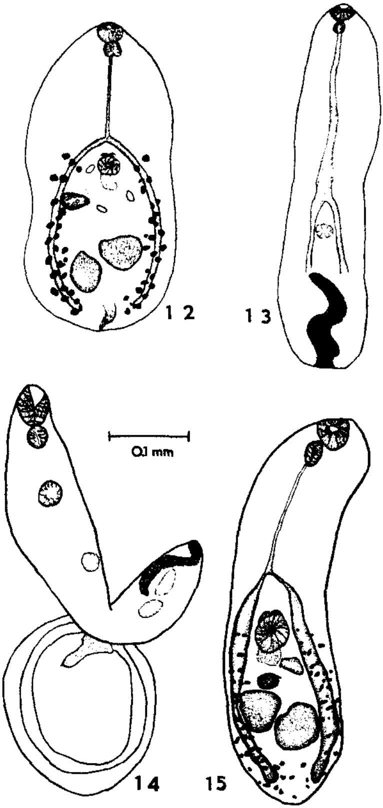
Metacercaria (Fig. 3)

The black pigmented cyst containing the metacercaria of *A. imperator* is easily seen in the trout. As in other members of the group, there is a primary cyst, hyaline and strong, within two outer layers. At least one of these is the host tissue surrounding the organism and is heavily pigmented. The primary cyst, 0.231 by 0.154 mm., is evenly oval in outline. The encysted metacercaria is folded upon itself and is very like the same form in the other heterophyids. The oral sucker, pharynx, traces of the oesophagus, and cuticular spines are distinguishable. The body contains many crystalline objects and others that resemble oil-droplets. The terminal area is filled with a mass of dark globules.

Efforts to cause excystment by artificial digestion failed with a single exception. This specimen probably represents a case of precocious development. All organs were present and there were three or four eggs in the uterus (Fig. 12). Other specimens freed by mechanical means never showed such advanced development. Rudimentary gonads, an S-shaped excretory sac filled with black material, and the faintly outlined acetabulum were usually present (Figs. 13 and 14).

The "black spots" occur on the ventral and lateral parts of the body on the skin just under the scales, and are frequent on the fins, tail, operculum, jaws, inside the mouth, on the gill bars, etc. (Table I). In only two fish, so heavily infected that they were almost uniformly black, were there cysts in the flesh, and even then only a few occurred. There seems to be no selected site for encystment such as Cameron found for *A. venustus* in the catfish.

In addition to Lakes Commandant and St. Bernard, infected trout have been reported from many lakes in the Commandant area. Curiously enough,



FIGS. 12 – 15. *Apophallus imperator sp. nov.* FIG. 12. *Precocious excysted metacercaria.* FIGS. 13 AND 14. *Excysted metacercariae.* FIG. 15. *Seven-day-old adult.*

of neighbouring lakes with apparently very similar characteristics and common outflow, observers have noticed that one will show infected trout and the other will be free. A small portion of trout skin sent by the Federal Department of Fisheries from a New Brunswick lake carried similar cysts. Hunter,

TABLE I

DISTRIBUTION OF HETEROPHYID BLACK SPOT IN TROUT

Body area	No. of cysts per sq. in.		
Base tail fin	54	43	34
Tail fin	61	116	10
Lateral line—dorsal fin	80	37	29
Dorsal fin	47	64	6
Pectoral fin	100	112	22
Base pectoral fin	80	50	21
Gill cover (external)	63	65	12
Gill cover (internal)		5	
Gill arch		4	3
Peduncle		10 (total)	7 (total)
Anal fin		50	7
Pelvic fin		40	10

These cysts were counted in three specimens selected at random. They were also found in the mouth, tongue, and eyes but were not counted there.

and Hunter and Hunter have twice reported black cysts on trout in New York State. In one instance from Lake Titus they refer to them as Strigeids (13). In the other, from Canopus Creek, they term them only "Black spots" (12). A rainbow trout (*Salmo irideus*) from the collection of the late Mr. Atkinson from the Lake Commandant area shows cysts similar to those of *A. imperator*. Whether these are the same parasite and identical with *A. imperator*, it is impossible to say, but the two are certainly very alike.

A paper presented by Linton (17) at the International Zoological Conference in 1907 is of interest in view of the present information. At that time he reported a black-spotting infection of trout from Alder Lake in the Catskills. The limited information and conditions he gives suggest that the metacercaria he found were not heterophyids. The cysts were larger than those of the present form, and he saw structures in the metacercariae that were probably oral and ventral suckers and holdfast organs. This fact, and the part apparently played by kingfishers, suggest that his material was of strigeid relationship. This supposition is further supported by the presence in Alder Lake of potential snail vectors of this family. One small metacercaria in which he noted only an oral sucker and a pharynx could possibly have been a heterophyid but this cannot be judged with any certainty.

All the speckled trout (*Salvelinus fontinalis*) caught from Lake Commandant were infected. The part other fish might play in maintaining an infection is of interest. Several yellow perch (*Perca flavescens*) and common suckers (*Catostomus commersonii*) (usually found associated with infected trout) failed to show any evidence of heterophyid infections. Similarly lake trout (*Cristivomer naymaycush*) seemed free. These fish were all carefully examined, then subjected to artificial digestion. No cysts were recovered.

The Egg (Fig. 2).

The egg is brownish yellow and oval in shape, usually narrowed at the operculated end and rounded at the other where a small protuberance often occurs. Variations to this general scheme are common. Size is fairly uniform, 0.028 by 0.16 mm. to 0.034 by 0.017 mm. Some are embryonated when voided by the host but all contain miracidia within three weeks. Hatching was not accomplished in the laboratory and mechanical rupture of the egg always destroyed the miracidium.

Experimental Investigations

An effort was made to identify the snail-vector. It has been shown previously that related genera require an operculate snail for development. By far the commonest snails in the infected waters are *Amnicola* sp., but exposure experiments with these snails and hatchery-raised trout have been unproductive. A small variety of *Campeloma* found in the lake was also used in this way with a similar failure. Trout and other fish were exposed to the snails of the non-operculate genus *Helisoma* taken from Lake Commandant. In this case the trout remained uninfected but black pigmented strigeid cysts developed in the other hosts.

In an effort to judge what might be the natural final host of this organism, various laboratory animals were fed cysts or infected fish (Tables II and III). The birds and rodents used had all been raised in the Institute. The cats came partly from the Poultry Department, Macdonald College, and partly

TABLE II

INFECTION TRIALS WITH EXPERIMENTAL ANIMALS

Host	Period of infection	Result of faecal examination	Result of post-mortem examination	Remarks
Cat	7 weeks	Positive	Adult *A. imperator* in small numbers	Cat fed eviscerated trout
Cat	7 days	—	Immature *A. imperator*	
Cat	10 weeks	Positive	—	Faecal examinations negative until fourth week and after tenth week
Cat	12 days	—	Adult *A. imperator* recovered	
Pigeon	7 weeks	Positive fifth week	Adult *A. imperator* recovered	Fed cysts only
Pigeon	12 weeks	Positive sixth week	Negative	
Duck	12 weeks	—	Negative	—
Duck	8 weeks	May have been positive at six weeks	Negative	A single egg was recovered from this duck
Duck	6 weeks	Negative	Negative	Fed filleted fish: (all the other birds were fed cysts only)
Chick	6 weeks	Negative	—	These chicks were 2 to 3 weeks old when fed cysts
Chick	5 weeks	Negative	Negative	
Heron	8 weeks	—	Negative	
Porcupine	4 weeks	—	Negative	Fed diced cyst-bearing fins

TABLE III

SMALL RODENT INFECTION TRIALS

Rodent	Interval between attempted infection and post mortem	Results			Remarks
		Intestine	Other organs	Faecal examination	
Guinea pig	5 weeks	Negative	Negative	Negative	Fed diced skin and fins, containing many cysts
Guinea pig	11 days	Negative	Negative	Negative	
Mouse	3 weeks	Negative	Negative	Negative	Fed cysts only
Mouse	2½ weeks	Negative	Negative	Negative	Fed diced infected skin
Mouse	2 weeks	Negative	Negative	Negative	Fed diced infected skin
Mouse	5 days	Negative	Negative	Negative	Fed diced infected skin
Mouse	3 days	Negative	Negative	Negative	Fed cysts only
Mouse	12 hours	Negative	Negative	Negative	Fed diced infected skin

from a known source at Kirkdale, Que. In no case had any experimental animals previously eaten fish.

In the experiments the intestine was examined in four sections, duodenum, remainder of small intestine in two equal portions, large intestine. No adult flukes were ever taken from the large intestine or duodenum, and most were found in the latter part of the small intestine.

The results of these attempts to infect various experimental hosts are notably irregular. They do indicate, however, that both mammals and birds may be parasitized by *A. imperator*, and that absence of eggs in the host faeces does not indicate freedom from infection. The appearance of eggs in faeces was considerably later than the earliest recovery of egg-bearing trematodes. The adult state is reached between the seventh and the twelfth days, and eggs first appear in the faeces almost a month later. Egg production was never very great, but no planned egg counts have been taken except to determine when they might be no longer present.

The table shows that the trematodes are not recoverable from the lumen or mucosa of the intestine after an interval. The exact length of this interval and the final fate of the parasites have yet to be determined.

The attempt to produce infections in small rodents was planned as an investigation preliminary to trials on the effects of heat on the encysted metacercariae, to determine the treatment necessary to make infected fish safe for human consumption. The results were uniformly disappointing. Fish scales were sometimes recovered, some of them still carrying the characteristic black pigment, but no cysts or freed trematodes were ever met with in the lumen or tissue of the intestine, or elsewhere in the animals.

The generic distinction recommended by Ciurea (10), based on the time required for development of egg-bearing adults, will not apply to *A. imperator*. He pointed out that members of the genus *Apophallus* were egg-bearing only

by the fourth day, while eggs could be demonstrated in those of the genus *Rossicotrema* by the second day after infection of the final host. Two extremes for egg-development represented by the writer's material are the metacercariae already mentioned, and non-ovigerous specimens taken from a cat on the eighth day after infection (Fig. 15).

In addition to these experimental hosts, the following fish-eating wild birds from the infected areas were examined with negative results, *Ardea herodias*, *Mergus merganser*, *Lophodytes cucullatus*, *Strix varia*. So far, members of the genus reported from Canada are *A. venustus* from various hosts, and *A. brevis* from the loon, *Gavia immer*.

Conclusions

Experimental observations, study of material, or analysis of literature on the genus *Apophallus* and related genera have shown that:

1. *Salvelinus fontinalis* in Quebec is the intermediate host of *Apophallus imperator* sp. nov., a heterophyid trematode.
2. *A. imperator* will become adult in the latter part of the small intestine of birds and mammals, experimentally in pigeons and cats.
3. The primary host is unknown. Snails of the genera *Amnicola*, *Campeloma*, and *Helisoma* from an infected area failed to cause the condition in trout in experimental trials.
4. The arrangement of the vitellaria, body-shape, position of testes, and period of development do not show adequate generic or specific characteristics in *Apophallus* and associated genera.
5. The position and number of gonotyls are of generic significance in the family Heterophyidae.
6. The nature of the gonotyls is of value in specific diagnosis within the genus *Apophallus*.

Acknowledgments

The author offers his very sincere thanks for the interested advice and support of the Director and staff of the Institute of Parasitology, and for the valuable assistance of various individuals and organizations in collecting materials. Mr. Frank Dale, other members and employees of the North Lake Fish and Game Club; Mr. L. J. Jorgensen and his staff at the Seigniory Club; Mr. Leslie Bates; members and staff of the St. Bernard Fish and Game Club, particularly Messrs. J. W. Mochon and J. MacMurray; Mr. B. W. Taylor and his assistants of the Hatcheries Branch of the Provincial Service of Game and Fisheries; Mr. Charles Fremont, of the same service, the Federal Department of Fisheries, and others, were generously co-operative. The donation of experimental animals by various individuals was also helpful.

The names of fish are those used by Hubbs (11), birds were identified following Taverner (26), and the names of snails are from the recent check list prepared by LaRocque (15).

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CANADIAN WILTSHIRE BACON

I. OUTLINE OF INVESTIGATION AND METHODS[1]

By W. H. Cook[2], N. E. Gibbons[3], C. A. Winkler[4], and W. H. White[3]

Abstract

This paper introduces an investigation involving bacterial, chemical, and physical measurements on bacon and pickle from 22 packing plants. The objects were to determine the over-all variation occurring in practice, the general sources of the variation, and the specific effect of certain curing practices. Methods employed for shipping the samples, sampling, and analyses are described.

It was found that small samples of curing pickle change slightly in nitrite and bacterial content during shipping periods approaching a week's duration, but that these changes are of negligible importance from a practical standpoint if the pickles are kept at temperatures near the freezing point.

Introduction

During recent years the export of bacon as Wiltshire sides* from Canada to Great Britain has assumed considerable importance in Canadian agricultural economy. As a result, investigation of the many problems associated with the manufacture and export of this type of bacon has become increasingly necessary. The present paper, introductory to a series forthcoming from these laboratories, outlines the scope of an investigation into a number of these problems, and describes the methods used for transporting the experimental sides and pickle from the packing plants to the laboratory, and the sampling and analytical methods employed.

The packing plants engaged in the export bacon trade in Canada are widely distributed and the period required for transporting the product naturally varies considerably. A survey of the methods used for making Wiltshire sides showed that, although the individual plants adhered quite closely to their own particular method, there was considerable variability in the practices

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 34 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 893.

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* *For a general description of Wiltshire sides, method of curing, and typical compositions of bacon and pickle, the reader is referred to the publications of the Food Investigation Board, London, England (1–4).*

adopted in different plants. This survey, however, gave little indication of the extent to which these variations affected the composition and quality of the bacon. Consequently, the present investigation was undertaken to determine: the over-all variation of the bacon with respect to the several measurements; the main general sources of variation; and more specific information on the effect of certain curing practices.

The over-all variation of Canadian bacon with respect to an individual property may be divided into: differences between sides from the same plant; differences between sides from different plants; and differences due to age from curing. The differences between sides from the same plant represent the combined effect of inherent differences in the curing quality of different carcasses and slight variations in the treatment each carcass may receive from the time of slaughter to the baling of the finished product. The difference between sides from different plants includes the sources of variation recognized above within factories, and also the additional effect of the different practices and pickle compositions used in different establishments.

Although a large number of factors may affect the composition and quality of bacon, particular attention has been given to the effect of the number of "stitches" (injections) per side used in pumping, the number of days in cure, and the composition of the pickles used. This approach to the problem was favoured, as curing is fundamental to bacon production. The particular factors mentioned above are apparently considered to be the most important, since each plant adheres closely to its chosen practice respecting them. Again, if necessary to improve quality, these factors could be modified promptly and inexpensively.

The relation between pickle and product is a function of the scale on which the curing practice is conducted, as shown by the fact that small pieces of pork "cured" in the laboratory (7) may have a salt content more than twice that of the factory product. If the results of investigations into the relations between pickle and product are to be applied in practice, it is essential that the materials studied be typical of those used or produced on a commercial scale. The practices followed in the 22 plants represented in this study varied sufficiently to give considerable information on the effect of pickle composition and other factors on the quality of the bacon.

It was necessary to employ statistical methods to interpret the large body of data obtained, while retaining certain confidential details. For details of the statistical methods used in this and subsequent papers the reader is referred to Snedecor (8).

Method of Shipping Samples

Both pickle and bacon are subject to chemical and bacteriological changes if held at ordinary temperatures, and special arrangements had therefore to be made for transferring these materials relatively unchanged from the plants to the laboratory. The methods employed are described below.

Bacon

The period of transport from the plants to the port of Montreal varies from less than one to six days. To minimize abnormal changes during transport, the experimental sides were shipped with regular export shipments in a refrigerator car to the docks at Montreal. Immediately a car was opened, the samples were transferred to a room at 0 to 2° C. When the entire shipment of samples had been accumulated it was transferred to the laboratories by refrigerated truck.

Pickle

Effect of Age and Temperature on Composition

Before undertaking these studies it was necessary to determine the conditions under which pickle may be shipped for periods of about five days duration without significant change in its chemical composition or bacterial content. In some preliminary experiments tank pickles, taken at the beginning, middle, and end of cure, were stored at 1.1 or 4.5° C. and at 25° C. for periods of about a week, in small sealed jars under aerobic conditions, since air was almost certain to be present in the test samples. Analytical results indicated that the chloride and nitrate content remained constant, while the nitrite content and bacterial numbers showed greater variation from day to day than could be accounted for by experimental error. This led to a more extensive study of the changes in nitrite content and bacterial numbers of pickles obtained from one plant at various stages of cure.

The changes in the nitrite content of five of the above pickles, representing the several types of change observed during storage at 25 and 4.5° C. or lower, are shown in Fig. 1, and the changes in the bacterial content in Fig. 2. The points in these figures represent the means of the determinations made at each sampling, and the ordinate of the cross-hatched section the difference necessary for statistical significance computed from the standard error of the replicated tests.

The nitrite content was determined colorimetrically by a procedure already described (9). The curves on the left-hand side of Fig. 1 were plotted from values obtained by comparing the colour developed in the sample with that of a standard solution of nitrite in a visual colorimeter. Those on the right-hand side were obtained with a photoelectric colorimeter, which yielded more precise results as shown by the smaller necessary difference.

Bacterial numbers were determined on nutrient agar and on 10% salt agar with incubation at 20° C. In Fig. 2 the ordinate represents the changes from the initial value during storage, these changes being expressed as the change in the logarithm of the bacterial number per ml. of pickle. The number of organisms observed on the medium containing 10% salt was much higher than that observed on salt-free media. The actual number present was not of particular interest in this study but will be discussed more fully in later papers of this series.

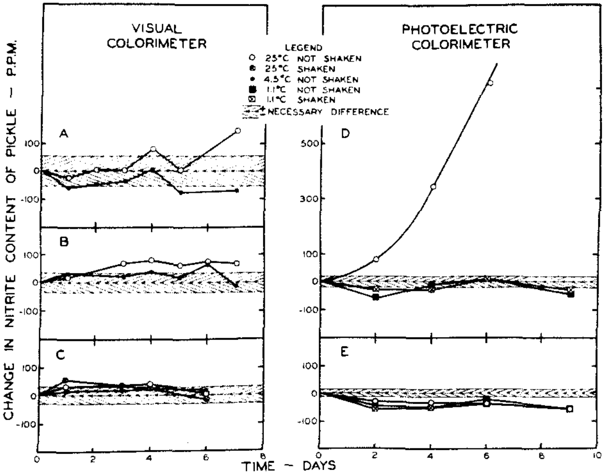

FIG. 1. *Changes in nitrite content of pickle during storage at 25° C. and 4.5° C. or lower.*

The curves in Fig. 1 show that storage temperature had little effect on the nitrite content of certain pickles (*C* and *E*), while others show a small (*A* and *B*), or decided (*D*), increase in nitrite content during storage at room temperature. Fig. 2 shows that the bacterial number observed on a salt-free medium remains relatively constant during storage at 4.5° C. or lower, but decreases during storage at 25° C. The counts observed on a medium containing 10% salt vary with time, but there is no consistent effect of temperature. These results showed definitely that the changes in nitrite content, and bacterial counts by certain methods, are reduced by keeping the pickle at a temperature of 4.5° C. or lower. Methods for maintaining these low temperatures during transport for periods up to five days will be described in the next section.

The results obtained with pickles *C*, *D*, and *E* (Fig. 1) and *D* and *E* (Fig. 2) indicate that shaking, comparable with that occurring in transport, had no appreciable effect on the nitrite content or bacterial numbers.

It is evident from Figs. 1 and 2 that even when the pickles are kept at temperatures of 4.5° C. or lower, the changes in nitrite content and bacterial numbers during storage may exceed the "necessary difference", or the day-to-day variation attributable to experimental error. The majority of these changes must therefore represent real alterations in composition. These

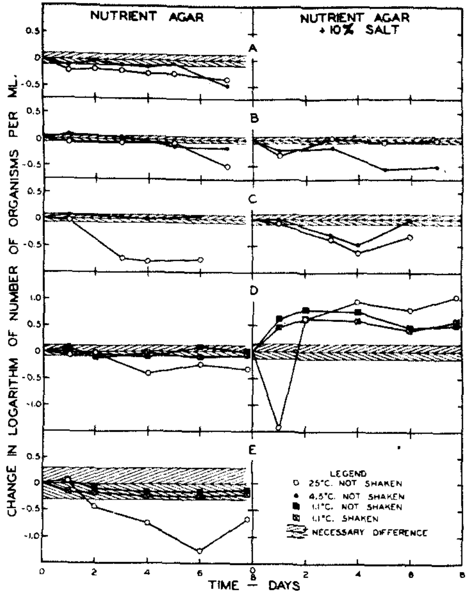

FIG. 2. *Changes in bacterial numbers in pickle during storage at 25° C. and 4.5° C. or lower.*

changes appear to be of two types: those showing a maximum or minimum during the storage period as shown by pickles *A* and *B* in Fig. 1, and *B* and *C* (10% salt medium) in Fig. 2; and those showing an increase or decrease only as shown by pickle *E* in Figs. 1 and 2. The analyses on these and other pickles failed to show any definite relation between the type of change and the stage of curing, i.e., whether the pickle was taken at the beginning, middle, or end of cure. Likewise the changes appear to be relatively independent of the original nitrite or bacterial content. It seems probable that some of the rather sudden changes in composition may be related to such phenomena as protein precipitation. It was therefore concluded that although pickles stored at low temperatures may show small but definite changes in composition, these changes depend on unknown properties of the individual pickle and are consequently unpredictable. Ingram's (5) conclusion that tank pickles may be shipped for periods up to 10 days at temperatures below 10° C.

without appreciable change in their nitrite content does not appear to apply generally.

The differential changes in different pickles during transport must therefore be recognized as an additional source of error, to be combined with the sampling and experimental errors. By obtaining two samples of pickle from each plant at different times, it was possible to compute an over-all estimate of the experimental error, errors due to differential changes during transport, and the error of sampling the tank. The significance of differences between the composition of pickles from different plants could therefore be assessed by comparison with the variance within plants at different times. It is possible that this latter value yields an exaggerated estimate of the error attributable to sampling, changes, etc., since it is almost certain that the composition of the original pickle from an individual plant varies, at least slightly, from time to time.

Although a decrease in the bacterial content of pickle, as indicated by growth on nutrient agar at 20° C., appears to be associated with an increase in nitrite content during storage at 25° C. in these experiments, little significance can be attached to this observation. The small containers used were by no means typical of the storage tanks employed in practice, and the experiments were not conducted on a sufficiently extensive scale to permit definite conclusions.

Containers for Maintaining Low Temperature During Transit

The results of the previous section showed the necessity for using containers that would maintain the pickles in the vicinity of the freezing point during shipment to the laboratories, i.e., for periods up to four or five days' duration.

Four types of containers were tested: (i) a common "thermos" bottle of half-pint capacity; (ii) an insulated commercial isothermal jug of one gallon capacity containing a half-pint jar of pickle and 7 lb. of ice; (iii) a gallon pail insulated with 1½ to 2 in. of cork and containing 4½ lb. of ice in addition to the pickle jar; and (iv) a 2-gal. can insulated with 2 to 2½ in. of wool felt and containing 15 lb. of ice. Typical results given in Table I show that the last container only was satisfactory, and this type was used throughout the investigation. The majority of the experimental samples were received at 0° C., a few at 5° C., and only an occasional one at 10° C. or higher.

Method of Sampling

Bacon

The 44 sides of Wiltshire bacon, submitted by the 22 plants, were sampled for analysis three times: (i) on receipt at the laboratories, representative of their condition when shipped from Montreal; (ii) after 10 days' storage at 1.1° C., representative of their condition on arrival in London, England; and (iii) after smoking for 14 hr. at 43 to 46° C., which yielded material approximately representative of that in the British retail store. English practice favours smoking periods of 36 to 48 hr. at relatively low temperatures

TABLE I

TEMPERATURE IN °C. OF PICKLE KEPT IN CONTAINERS OF VARIOUS TYPES

Period of exposure at 25° C., days	"Thermos" bottle 1 pint capacity	Insulated commercial isothermal jug, containing ½ pint pickle + 7 lb. ice	Gallon pail insulated with 1½ – 2 in. cork dust, containing ½ pint pickle + 4½ lb. ice	2-gal. can insulated with 2 in. wool felt, containing ½ – 1 pint pickle + 15 lb. ice
0	2.3	0.2	0.0	0.0
1	12.5	1.1	0.0	0.0
2	17.8	16.7	1.0	0.0
3	21.0	—	11.1	0.0
4	22.5	—	19.7	0.0
5	—	—	—	12.5

compared with Canadian methods. (In these studies the ham was the only portion of the side to be smoked. For this and other reasons the smoking period was reduced.)

On receipt at the laboratory the sides were placed in a room at 4.5° C. and all sampling was done at this temperature. The sides were unwrapped and samples for determining the bacterial number taken first. After removal of material for the chloride, nitrate, nitrite, moisture, pH, colour, colour stability, and tenderness determinations, and measurement of the oxidation-reduction potential of the whole meat in the ham, the sides were rebaled and transferred to a room at 1.1° C. After 10 days' storage the bales were again opened and sampled for these determinations, with the exception of tenderness measurements. This completed the sampling for bacterial numbers, chloride, pH, and oxidation-reduction determinations, but the ham from each side was smoked and again sampled for the other determinations.

Samples were also taken from the sides for studies on salt distribution, the effect of heat treatments comparable with smoking, and the fats. The samples used for these experiments will be described in the papers reporting the results.

The exact position from which the samples were taken and certain details of sampling can best be described by reference to Fig. 3. Samples of the pleural membrane over the second and third, and over the ninth and tenth ribs were removed for making bacterial counts at each sampling. At the first sampling the fourth and fifth ribs were removed without contamination of the pleural membrane and stored separately in an atmosphere of 95% relative humidity at 1.1° C. for a longer period than the sides were stored, to determine the surface bacterial number when visible slime became evident.

Material for the other determinations was obtained after the ham was removed, as indicated in Fig. 3. Samples for chemical analysis and the pH measurements were obtained by cutting a slice from the ham, while a triangular section removed from the side provided material for colour, colour stability,

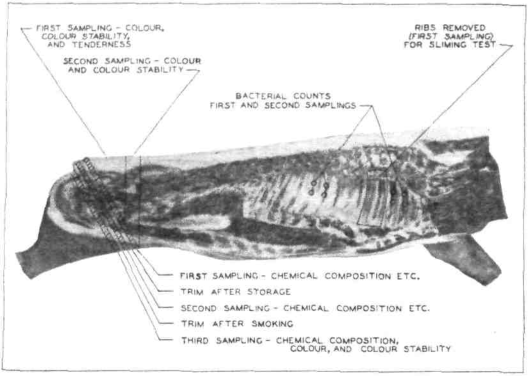

FIG. 3. *Portions of Wiltshire side used for determining bacterial counts, chemical composition, and physical properties at each sampling.*

and tenderness measurements. Oxidation-reduction potential was obtained by inserting a small pointed platinum electrode into the ham after removal of the slice for chemical analysis.

After storage the cut surface of ham was trimmed and another slice removed for analysis. At the same time a piece was removed from the side for colour and colour stability measurements. Oxidation-reduction potentials were again taken as described above. After smoking the ham was trimmed and another slice removed for both the physical and chemical measurements.

There are certain advantages and disadvantages in this more or less systematic method of sampling. Apart from certain practical limitations, it seemed desirable, since this study was concerned primarily with variations within and among plants, to take the samples from all the sides, at a given sampling, from comparable regions. This should permit more precise comparisons between the compositions of the same portion of different sides than if the samples had been taken at random from the whole side, or a preselected part of it. This procedure, however, sacrifices precision in determining the effect of ageing, for although the portions taken from each side at a given sampling are comparable, those taken at different samplings differ systematically in both time and position. In making the statistical analyses it is possible to determine the variance attributable to "between samplings" but it cannot be stated with assurance whether such differences represent a real change with time, or a difference between positions that existed originally. This subject will be discussed further in relation to the results obtained.

The slices of ham taken at each sampling for chemical analysis were immediately trimmed free of fat, bone, and connective tissue. Three small samples representing the outside, centre, and inside of the slice were removed for a study of salt distribution to be described in a later paper. The remainder of the lean meat was ground and thoroughly mixed by passing it through a food chopper several times. The pH of this ground material was immediately determined with a glass electrode, after which it was placed in a moisture-tight sample bottle, frozen in a room at −29° C. and held at this temperature until required for analysis. Storage in the frozen state usually did not exceed a few days. The samples were thawed by placing them in a room at 4.5° C. for a few hours. They were then mixed thoroughly and portions for analysis weighed out in the same cold room. The sample residues were refrozen immediately to preserve the material for any determinations that required to be repeated. Extreme care was necessary at this stage since ground material kept at 4.5° C. for more than a few hours showed a significant increase in nitrite content, and this change was accelerated by exposure to higher temperatures.

All the above measurements were objective, and no attempt was made to estimate subjective qualities such as flavour. A special study of this important attribute of quality has been undertaken.

Pickle

Two distinct pickles are used for curing Wiltshire bacon. One of these, designated "pump" pickle, is injected into the sides before they are placed in the curing vats, while the other, designated "tank" pickle, is used to cover the sides. Tank pickle suffers progressive changes in composition during the curing period. In order to obtain some estimate of the magnitude of these changes a sample of the tank pickle was taken when the sides were put in to cure, and again when they were removed. These two samples are subsequently designated "cover" and "spent" pickle respectively. When an estimate of the composition of tank pickle was necessary for certain computations, this was taken as the average composition of the cover and spent pickle with respect to the constituent in question.

Corresponding samples of pump, cover, and spent pickles were obtained twice from each plant with an interval of about a month between samplings. The second set of samples was taken from the pickles used for curing the bacon from which the experimental sides were obtained. In view of the small difference observed between successive pickle samplings, the mean composition of the two pickles was used for studying the relations between pickle and product in order to reduce the obscuring effect of experimental errors. The results of certain determinations on the pickles were lost or excluded as a result of errors in sampling, failures in transport, or losses in the laboratory.

When the pickle samples were received they were unpacked and placed in a room at 0° C. until the analyses were complete. Samples for bacterial numbers were removed to sterile glassware, plated, and incubated imme-

diately. Sufficient material for the chloride, nitrate, nitrite, pH, and oxidation-reduction potential measurements was removed to an ordinary laboratory for analysis and these determinations were made at room temperature. As the proteins tend to precipitate from pickle at room temperature the samples for this determination were pipetted from the original sample at 0° C. The remainder of the sample was filtered and colour measurements made in a room at 4.5° C.

Methods of Analysis

The methods employed for making the bacterial counts on both bacon and pickle will be described in later papers reporting the results of the measurements. The procedures followed in determining chloride, nitrate, and nitrite have already been described (9).

The protein nitrogen content of the pickle was determined on suitable portions (usually 10 or 25 ml. depending on the protein nitrogen content) pipetted into Kjeldahl flasks in the cold room. Nitrate nitrogen was removed (6, p. 27) before the Kjeldahl digestion.

The moisture content of the bacon was determined on 2- to 3-gm. portions of the ground material, by drying in flat aluminium dishes to constant weight (16 to 24 hr.) at 100° C. Preliminary measurements at lower temperatures yielded the same results, within experimental error, on this relatively fat-free material, but a much longer drying period was required. Actually it was difficult to attain a truly constant weight at any temperature, but after drying for 16 to 24 hr. at 100° C. the loss of weight over an additional 4-hr. drying period was never more than the equivalent of 0.05% moisture. Differences of this magnitude were smaller than the error between duplicates, and considerably less than the over-all sampling error.

The pH and oxidation-reduction potential measurements were made with a Bechmann pH meter. The appropriate standard electrodes supplied with the instrument were used for the determinations on pickles. The pH measurements on bacon were made in a room at 4.5° C., using a large glass electrode and extension leads. Both the glass and calomel electrodes were forced into a portion of the ground sample. Readings were required to check within 0.05 pH at different positions in the sample. Standard buffer solutions were used for adjusting the instrument at this low temperature, and all reported values were corrected to 20° C.

The oxidation-reduction potential of the bacon was measured with a special platinum electrode consisting of a 60° platinum cone, ¼-in. diameter at the base, carried on a ¼-in. diameter bakelite tube. This electrode was inserted to a depth of 2 to 2½ in. into the ham and the circuit completed by inserting the calomel electrode into a cut at the surface. The observed potentials were converted to Eh by correcting for the potential of the calomel electrode at 4.5° C. The instrument and electrodes used for the Eh measurements were checked with a buffer solution of known pH containing quinhydrone. Several difficulties were encountered in making the Eh measurements in both bacon

and pickle, and there was some uncertainty in the results obtained. These will be discussed more fully when the results are presented in a later paper.

The colour and tenderness measurements on the bacon were made by means of instruments and methods already described (10, 11). These observations were made on slices or portions of whole meat, immediately after cutting, in a room at 4.5° C. Samples were chosen that were free from obvious streaks of fat or connective tissue. An estimate of colour stability was obtained by repeating the measurements on these slices periodically during exposure to air at a temperature of 10° C. and a relative humidity of 95%. This condition avoided serious drying of the samples, which has been shown to affect the colour (12). The results therefore indicate the stability of the colour to atmospheric oxidation at a temperature commonly prevailing in the storage chamber of a retail store.

Acknowledgments

The authors wish to express their appreciation and thanks to the management and staffs of the packing companies for their cordial co-operation in providing certaiu confidential details relative to their curing practice, for donating the bacon and pickle for analysis, and for collecting and forwarding these samples. These include: Burns and Co. Ltd., at Calgary, Edmonton, and Prince Albert; Canada Packers Ltd., at Toronto, Peterborough, Hull, Montreal, and Edmonton; Dumarts Ltd.; F. W. Fearman Co. Ltd.; First Co-Operative Packers of Ontario; Fowler's Canadian Co.; Gainers Ltd.; J. M. Schneider Ltd.; Swift Canadian Co. Ltd., at Toronto, Winnipeg, Moose Jaw, and Edmonton; Wellington Packers Ltd.; Whyte Packing Co.; Wight and Co. Ltd.; and Wilsils Ltd. In addition the following firms furnished pickle and all other information requested but were unable to supply bacon at the time this phase was investigated: Burns and Co. Ltd., at Regina and Winnipeg; Canada Packers Ltd., Winnipeg; and Union Packing Co., Calgary. Special thanks are tendered to the management and staff of Canada Packers Ltd., Montreal, for assisting in the collection at Montreal of the bacon from all plants and its transport to Ottawa, and of Canada Packers Ltd., Hull, for smoking the test material and assisting with the sampling. It would have been impossible to conduct the investigation without the whole-hearted co-operation of these firms.

Thanks are also tendered to the officers of the Dominion Department of Agriculture, particularly those of the Marketing Services, for assistance in making the general arrangements, and to that Department for defraying the cost of shipping the samples.

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CANADIAN WILTSHIRE BACON

II. CHLORIDE, NITRATE, AND NITRITE CONTENT OF BACON AND PICKLE[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

Analysis of Wiltshire bacon produced in Canadian plants showed that different sides varied in their chloride, nitrate, and nitrite contents, the chloride content being the most uniform. Although the observed variations were statistically significant they do not necessarily affect the quality of the product. An analysis of variance showed that differences between the sides from different plants were the main general source of variation in the chloride and nitrate contents, while the differences between sides from the same plant were the main source of variation in nitrite content. This, and other evidence, indicates that the properties of the individual side affect its nitrite content to a considerable extent.

The variation in the composition of the pickles used in different plants was significantly greater than the variations within plants, although the chloride content was relatively uniform throughout. Other curing practices, such as the number of injections used for pumping a side and the time in cure, also varied between different plants. Statistical computations showed that the number of injections used for pumping was correlated with the chloride and nitrite content of the sides, while their nitrate content was correlated with the nitrate content of the pump pickle. Although these factors affected the composition of the product with respect to each constituent, the level of the correlation coefficients was rather low. It is therefore concluded that most of the observed variation in the bacon was contributed by other unmeasured factors, or by inherent differences between the carcasses.

The analysis of variance showed significant differences between the content of the three constituents at different samplings. The method of sampling, however, did not permit the true effect of ageing to be distinguished precisely from the effect of systematic differences in position, and the observed differences between samplings might possibly have been due entirely to the effect of position.

Introduction

Although the quality of bacon may be affected by many factors, relatively few of these can be closely controlled. Those that can be standardized and reproduced from time to time in a given plant include the composition of the pickles with respect to the several salts used, and certain of the curing processes. Different plants, however, use different pickle formulas and curing practices, and this, together with the fact that the composition of the product may depend on other factors that are not closely controlled, can give rise to certain variations in the product. This paper reports the chloride, nitrate, and nitrite contents of the bacon, the general sources of variation, and more specifically, the effect of pickle composition and certain curing practices.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 35 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 894.

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An outline of this investigation and a description of the methods used have been reported in the first paper (5) of this series. Statistical methods (7) were employed to reduce the large body of results, and permit their interpretation in terms of the required information.

The general mean and the standard deviation of a single observation served to describe the composition and over-all variation of the bacon and pickle with respect to each of the constituents. The coefficient of variability was computed from these quantities to facilitate comparison of the relative variability of different constituents. The practical significance of the observed variations had naturally to be assessed from other considerations.

The principal sources of variation were determined by an analysis of variance. For bacon, the total variance could be subdivided into portions attributable to: average differences between sides from different plants; differences between sides within plants; average differences between samplings; and sampling and analytical error. Although all the analyses of the samples were made in duplicate, it was impossible, within the limits of available time and material, to obtain and analyse two independent pieces from each side at each sampling. In consequence the residual variance was used to estimate the combined sampling and analytical error.

The results obtained with pickle were analysed in the same way except that only two sources of variance could be recognized, namely, within and between plants. For reasons already given (5) the variance within plants had to be taken as an estimate of the sampling and other errors. This variance probably overestimates these errors, since the samples for each plant were obtained at different times, and consequently the observed differences include any real change in the composition of the pickle from time to time.

The specific effect of pickle composition, number of stitches per side used in pumping, and the number of days in cure, on the concentration of salts in the product, was determined by computing simple correlation coefficients between the quantity of a given constituent in the bacon, the number of stitches per side, the number of days in cure, and the quantity of the same constituent in the pump and tank pickles. In some instances it was possible to combine two of these quantities. For instance the amount of salt contributed by the pump pickle may reasonably be taken as the product of its salt content and the number of stitches used per side. This assumption appears to be valid, since results by Callow (3, pp. 65–70) indicate that the amount of pickle retained is proportional to the number of stitches. Nevertheless some uncertainty remains, since the pumping equipment and method in different plants may not inject the same amount of pickle per stitch, while the number of stitches in the ham portion analysed may not remain in a fixed proportion of the number per side. Similarly the product of the chloride content of the tank pickle and the number of days in cure were used for computing certain correlations.

Finally the composition of the bacon will be affected by the composition and method of application of both the pump and tank pickles. The relative

effect of these two phases of curing cannot be established directly from simple correlation coefficients, since certain factors in each phase may be associated. Thus a plant using a weak pump pickle may also use a weak tank pickle, or a plant accustomed to the production of a mild bacon may reduce both the number of stitches used in pumping and the number of days in cure. By computing partial correlation coefficients, however, it was possible to determine the effect of the pumping practices independent of tank curing practices and vice versa. These computations were made wherever the simple correlations were significant, in order to establish the observed relation independent of associated factors.

Chloride

The mean chloride content of the bacon and pickles from all plants, over all samplings, appears in Table I. The average chloride content of 3.9% and a maximum of 5.8% indicate that Canadian Wiltshire is milder, on the average, than British bacon, if published results (1, 2) are typical of British curing practice.

The chloride content of the pickles is expressed on a weight-volume percentage basis, i.e., grams of sodium chloride per 100 ml. of pickle, or approximately lb. per 10 gal. The figures in Table I show that the mean chloride content of pump pickle approaches saturation (about 31.7% on a weight-volume basis at cellar temperature). Tank pickle is somewhat less concen-

TABLE I

CHLORIDE CONTENT OF BACON AND PICKLE

(As sodium chloride)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	3.93	29.20	28.32	23.80
Standard deviation, %	1.05	1.71	2.67	1.93
Coefficient of variability	26.79	5.86	9.41	8.10

Analysis of Variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.206	19	0.795	16	0.824	16	1.280
Between samplings	1	21.315**						
Between sides within plants	22	0.476*						
Between plants	21	2.652**	(18)	5.178**	15	13.815**	15	6.323**

* *Indicates 5% level of significance, in this and subsequent tables.*
** *Indicates 1% level of significance, in this and subsequent tables.*

trated initially and the chloride content naturally decreases during cure, cf., values for cover and spent pickles respectively. Although the greatest absolute variations in chloride content occur in cover pickle, the chloride content of the bacon is relatively more variable than that of the pickles.

The results of analyses of variance are given in the lower part of Table I. Those for bacon show that, on the average, both the variance between samplings from the same side and that between different sides from the same plant were significantly greater than the sampling and analytical errors. The variance between sides from different plants, however, was significantly greater than that between sides within plants. The significance of the variance between samplings will be discussed more fully in a later section. Although it must be recognized that sides cured at the same time in the same tank differ significantly in chloride content, it is obvious that the major portion of the variation is contributed by the different chloride contents of bacon from different plants.

The simple and partial correlation coefficients between the chloride content of the bacon and the chloride contents of the pump and tank (mean of cover and spent) pickles, the number of stitches per side used in pumping, and the number of days in cure, appear in Table II. The simple correlation coefficient between the chloride content of the bacon and the number of stitches used per side is the only one that exceeds the 5% level of significance. In fact, the coefficients between the chloride contents of the bacon and that of both pickles, although insignificant, are negative. This indicates that the chloride content of the bacon is not affected by the observed variations in the chloride content of the pickles used, but depends entirely on other factors.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE CONTENT OF PICKLE AND CHLORIDE CONTENT OF BACON

(As sodium chloride)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean chloride content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.45*
Days in cure	20	−0.33
Chloride in pump pickle (mean)	19	−0.36
Chloride in tank pickle (mean)	19	−0.10
Loss of chloride from tank pickle during cure (mean)	19	−0.33
Chloride in pump pickle (mean) × stitches per side	19	0.40
Chloride in tank pickle (mean) × days in cure	19	0.25
Chloride in pump pickle (mean) × stitches per side, independent of chloride in tank pickle (mean) × days in cure	18	0.41
Chloride in tank pickle (mean) × days in cure, independent of chloride in pump pickle (mean) × stitches per side	18	0.28

The simple and partial coefficients between the chloride in the product and such combined quantities as chloride in pump pickle times the number of stitches, and chloride in tank pickle times days in cure, were insignificant.

Although these results indicate that the number of stitches used for pumping a side is more important than any of the other factors studied, the level of the correlation coefficient, although significant, is so low that less than 20% of the observed variance in the chloride content of the bacon can be accounted for by variations in the number of stitches used. In consequence the major portion of the observed variation in chloride content, including that between sides from the same plant, must be due to unmeasured quantities concerned with curing, or to inherent differences between carcasses. Fortunately the over-all variation observed cannot be regarded as having a serious direct effect on the quality of the product, although it may be desirable to produce bacon of more uniform chloride content.

Nitrate

The mean nitrate content of the bacon and pickles, the standard deviation from these means, the coefficients of variability, and the results of an analysis of variance are given in Table III. The nitrate contents of the pickles are, on the average, comparable with those used in British practice (3, pp. 65–70) and would appear to be satisfactory. The nitrate content of both the product and the pickles is much more variable than their chloride content, as shown by the coefficient of variability. The relatively greater variation observed

TABLE III

NITRATE CONTENT OF BACON AND PICKLE

(As sodium nitrate)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	0.184	2.32	1.32	0.92
Standard deviation, %	0.220	2.469	0.923	0.644
Coefficient of variability	119	107	69.7	70.4

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	0.0125	19	0.342	16	0.380	16	0.056
Between samplings	2	0.0534*						
Between sides within plants	22	0.0228						
Between plants	21	0.2212**	18	12.173**	15	1.354**	15	0.798**

in the nitrate content of the bacon and pump pickle, as compared with that of the cover and spent pickles, was contributed largely by one plant that used much more nitrate than the average in its pump pickle. This practice had a *direct effect on the nitrate content of the product.*

The analysis of variance shows that there was a significant difference in the nitrate content between samplings. This phase will be discussed later. The difference between sides from the same plant did not exceed the analytical and sampling errors significantly. The difference between both the bacons and pickles from different plants, however, was significantly greater than the observed differences within plants.

The simple and partial correlation coefficients between the nitrate content of the bacon, and the known factors in the curing practice appear in Table IV. These correlation coefficients were computed on two bases, namely, including and excluding the results obtained from the plant using high nitrate concentrations in its pump pickle. By including the results from this plant, the correlation coefficients are based on the figures from which the results in Table III were computed. By excluding the results from this plant it was felt that the correlation coefficients were more typical of the effect of the ordinary variations in nitrate content of the pickles. As can be seen from the table, the exclusion of these results tends to reduce the degree of correlation between the nitrate content of the meat and quantities including the composition of the pump pickle, and to increase that with quantities involving the composition of the tank pickle.

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN NITRATE CONTENT OF PICKLE AND NITRATE CONTENT OF BACON

(As sodium nitrate)

Quantities correlated	Simple correlation coefficients			
	All plants		Exclusion of one exceptional plant	
	D.f.	*r*	D.f.	*r*
Mean nitrate content of bacon over all sides and samplings with:				
Pump stitches per side	20	0.13	—	—
Days in cure	20	0.29	—	—
Nitrate in pump pickle (mean)	19	0.92**	18	0.48*
Nitrate in tank pickle (mean)	19	0.59**	18	0.62**
Loss of nitrate from tank pickle during cure (mean)	19	0.09	—	—
Nitrate in pump pickle (mean) independent of tank pickle (mean)	18	0.88**	17	0.29
Nitrate in tank pickle (mean) independent of pump pickle (mean)	18	0.11	17	0.51*
Nitrate in pump pickle (mean) × stitches per side	19	0.89**	18	0.63**
Nitrate in tank pickle (mean) × days in cure	19	0.55**	18	0.57**
Nitrate in pump pickle (mean) × stitches per side, independent of nitrate in tank pickle (mean) × days in cure	18	0.80**	17	0.48*
Nitrate in tank pickle (mean) × days in cure independent of nitrate in pump pickle (mean) × stitches per side	18	0.02	17	0.39

Considering the coefficients given in the last column of Table IV it is evident that the nitrate contents of both the pump and tank pickles affect the nitrate content of the sides. This was to be expected, since the variation in the nitrate content of the two pickles was proportionately far greater than the methods used for their application. The partial correlation coefficients between the nitrate content of the bacon and that of the pump pickle, independent of tank pickle and vice versa, indicate that the nitrate content of the tank pickle has a greater influence on the composition of the product than that of the pump pickle. Nevertheless, the combined quantities, representing the known values of the pumping and tank-curing practices respectively, both yielded simple correlation coefficients that were highly significant. This suggests that the number of stitches per side and the number of days in cure, as well as the nitrate content of the two pickles, have some effect on the nitrate content of the sides. Partial correlation studies indicate that the composition of the pump pickle and the stitches per side are more influential than the composition of the tank pickle and the number of days in cure.

It is concluded from these results that the nitrate content of the pump and tank pickles and the number of stitches used in pumping are the principal factors affecting the nitrate content of the sides. It seems probable that, if more uniform pickle compositions and pumping practices were used in all plants, the relative variability of the product with respect to nitrate would be reduced to about the same level as that reported for chloride (Table I).

Nitrite

The mean nitrite content of the bacon and pickles, the variation of each, and the main sources of this variation are shown in Table V. The bacon con-

TABLE V

NITRITE CONTENT OF BACON AND PICKLE

(As sodium nitrite)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, p.p.m.	26.3	303	536	482
Standard deviation, p.p.m.	24.1	366	376	293
Coefficient of variability	91.6	121	70.2	60.7

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	127.8	19	8,331	16	6,551	16	3,966
Between samplings	2	818.6**						
Between sides within plants	22	1,213**						
Between plants	21	1,743	18	266,669**	15	285,593**	15	172,802**

tained 26 p.p.m. of sodium nitrite on the average. This is well within the 200 p.p.m. of sodium nitrite permitted by the Canadian pure food regulations (6). Although the coefficient of variability shows, on the average, considerable variation in the nitrite content of different sides, none of the sides, at any of the samplings, had a nitrite content approaching the legal limits.

The composition of the pickles with respect to nitrite content appears to be quite typical (3, p. 65–70), although these pickles were analysed during the summer months when the bacterial activity, and consequently the nitrite contents, would probably be maximal. On the whole the nitrite content shows about the same relative variability as the nitrate content.

The analysis of variance for bacon indicates that the difference between samplings and between sides from the same plant is responsible for most of the variance. In spite of the highly significant variations in the nitrite content of the pickles used in the different plants, the variance between the nitrite content of the bacon from different plants was not significant. This result is partly accounted for by the relatively large variance between sides within plants, most of which was contributed by the sides received from three or four plants. Detailed inquiry and examination of the results showed no valid reason for excluding these sides. The present investigation therefore indicates that the difference between sides treated in the same way is the main source of variation in nitrite content. More extensive analyses now under way, however, indicate that there is a significant difference between the nitrite content of sides from different Canadian plants after smoking in England. This cannot be taken as contradicting the present findings, since it will be shown later that the conditions used for smoking bacon in England may result in a differential development of nitrite in different sides.

The correlation coefficients between the nitrite content of the bacon and the known curing practices appear in Table VI. The correlation between the nitrite content of meat and the number of stitches used for pumping a side is the only one that is significant. Since correlations between the nitrite content of the bacon and that of the pump pickle never approach the level of significance, it appears that the number of stitches used for pumping increases the nitrite content of the bacon indirectly, rather than by a direct contribution of nitrite to the sides. This hypothesis is supported by the highly significant partial correlation between the number of stitches per side independent of nitrite in pump pickle. The possible nature of such an indirect effect is obscure, but it may result from the introduction of air or bacteria into the meat during pumping. It will be shown in a later paper that the pump pickles contained a considerable number of aerobic bacteria.

If nitrites can be produced from nitrates either on or within the sides after curing, it is evident that the observed nitrite content of the sides reflects both the extent or rate of this reaction as well as that between nitrite and haemoglobin, or muscle proteins. An attempt was therefore made to determine which of the two reactions was the more important in determining the nitrite content of the bacon. This was done by computing the nitrate : nitrite

TABLE VI

COEFFICIENTS OF CORRELATION BETWEEN NITRITE CONTENT OF PICKLE AND NITRITE CONTENT OF BACON

(As sodium nitrite)

Quantities correlated	Simple correlation coefficients	
	D.f.	*r*
Mean nitrite content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.57**
Days in cure	20	0.29
Nitrite in pump pickle (mean)	19	0.25
Nitrite in tank pickle (mean)	19	0.25
Loss of nitrite from tank pickle during cure (mean)	19	0.21
Nitrite in pump pickle (mean) independent of stitches per side	18	0.21
Pump stitches per side independent of nitrite in pump pickle (mean)	18	0.57**
Nitrite in pump pickle (mean) independent of nitrite in tank pickle (mean)	18	0.15
Nitrite in tank pickle (mean) independent of nitrite in pump pickle (mean)	18	0.15
Nitrite in pump pickle (mean) × stitches per side	19	0.34
Nitrite in tank pickle (mean) × days in cure	19	0.23
Nitrite in pump pickle (mean) × stitches per side, independent of nitrite in tank pickle (mean) × days in cure	18	0.27
Nitrite in tank pickle (mean) × days in cure, independent of nitrite in pump pickle (mean) × stitches per side	18	0.12

ratio in the pickles and bacon from the individual plants. It was felt that if this ratio was significantly lower in the bacon than in the pickle, production of nitrite in the sides would be indicated, while a higher ratio in the bacon would suggest that reactions favouring the disappearance of nitrite predominated. Moreover, the use of the ratio would tend to minimize the effect of systematic variations in the composition of the meat between the different positions.

These ratios were found to be extremely variable both in the bacon and in the pickle. When they were subjected to an analysis of variance, neither the difference between samplings, between sides from the same plant, or between sides from different plants were significantly greater than the residual variance attributable to sampling and analytical error. The nitrate: nitrite ratio in the tank pickle only showed significant differences among plants. In fact, the mean ratio for the bacon over all samplings and sides did not differ significantly from that of the pickle over all samplings and plants.

It was observed, however, that two sides from each of two plants had a very low nitrite content at the first sampling, and since the nitrate content was about average, the nitrate : nitrite ratio was exceedingly large. The nitrite content at the later samplings, however, had increased considerably, indicating the production of nitrite within these sides. Since the different

sides in this group showed considerable variation in nitrite content at all samplings it was necessary to test the significance of the observed differences. For doing this the nitrate : nitrite ratio was used in preference to the nitrite content, for reasons already given.

Since the mean nitrate : nitrite ratio of these four sides varied widely both between sides and samplings, with the variance approximately proportional to the mean, it was necessary to use the logarithm of these ratios in making the analysis of variance in order to provide valid tests (4) of significance. The results of this analysis appear in Table VII, from which it is evident that the differences between sides and samplings are both significantly greater than the residual variance.

TABLE VII

ANALYSIS OF VARIANCE OF SAMPLES HAVING LARGE NITRATE : NITRITE RATIOS INITIALLY

Figures on basis of logarithms of ratios

Variance attributable to	Degrees freedom	Mean square
Between sides within times	3	2.01*
Between times (samplings)	2	2.62*
Residual	6	0.412

It is concluded from these results that sides having a high nitrate : nitrite ratio initially, i.e., low nitrite content, may increase in nitrite content during maturation and smoking. Furthermore, the difference between sides indicates that the extent of this increase depends on the properties of the individual sides. Whether the variable increases in the nitrite content of different sides were due to varying bacterial loads or to inherent differences in the carcasses themselves is not known. Since this behaviour was not observed over all samples it appears that under average conditions nitrite production does not occur to any significant extent in samples containing an average nitrite content initially.

Difference Between Samplings

Certain changes, termed maturation, occur in bacon after removal from cure. Although these are believed to be beneficial to the general quality and flavour of the product (3, pp. 70–72), their nature is obscure. It therefore seemed desirable to consider the changes that occurred in all of the individual constituents and properties measured. The results given in previous tables showed that the three constituents dealt with in this paper did differ significantly between samplings. However, as pointed out in the first paper (5), the difference between samplings includes the effect of systematic differences between the positions from which successive samples were taken as well as the true effect of ageing and smoking. Since certain constituents, such as chlorides, should not suffer any change with time, these can be used as reference substances for assessing the significance of observed differences in other constituents which might change, e.g., nitrites.

Table VIII shows the mean chloride, nitrate, and nitrite contents over all sides by samplings, the difference between these means, and the significance of the differences compared with the sampling and analytical error. All three constituents showed a significant increase between the first and second samplings. The chloride content was not determined at the third sampling. Between the second and third samplings, the nitrate content remained practically constant, and the nitrite content decreased significantly. The increased chloride content must represent the effect of position or chloride distribution, which will be discussed in a later paper. A similar conclusion must be reached for nitrate since there is no evidence that any appreciable quantity was converted to nitrite. Since the increase in nitrite between the first and second samplings is of the same order on a percentage basis as that observed with chloride and nitrate, it appears that this increase represents the effect of position rather than time. The decrease in nitrite between the second and third sampling may indicate a decrease in nitrite during smoking, since the nitrate content remains practically constant.

TABLE VIII

DIFFERENCE BETWEEN SAMPLINGS

Constituent	Mean by samplings			Difference between means		
	First (1)	Second (2)	Third (3)	1—2	2—3	1—3
Sodium chloride, %	3.43	4.42	—	0.985**	—	—
Sodium nitrate, %	0.144	0.204	0.205	0.060*	0.001	0.061*
Sodium nitrite, p.p.m.	22.6	31.1	25.2	8.42**	5.83*	2.59

A further attempt was made to determine the effect of age on nitrite content by computing simple correlation coefficients between the nitrite content and the age of the bacon at the three samplings independently, and over all analyses irregardless of samplings. These were found to be -0.04, -0.20, and -0.28, for the first, second, and third samplings respectively, and $+0.02$ over all samplings. None of these coefficients is statistically significant, although the negative sign within samplings indicates a decrease in nitrite with time. When the computation is made over all samplings, the effect of position intervenes, decreasing the correlation coefficient and changing its sign.

In conclusion there is no evidence to indicate that, on the average, a serious change occurs in the nitrite content of bacon during storage at 1.1° C., or smoking for 14 hr. at about 45° C. There is some indication that nitrite may be formed in sides having a low nitrite level on removal from cure. The difference between the nitrite contents of sides cured in the same plant suggests that much of the observed variability is due to some factor that is not closely controlled in commercial practice. This factor may be some inherent property of the individual sides, or differential bacterial contents.

Difference Between Bacon and Pickle from Different Plants

The results reported in earlier tables show that although the differences between sides from the same plant were generally significant, the differences between sides from different plants were usually greater. It is therefore of interest to determine how the concentrations of the several salts in the sides and pickles from the various plants are distributed around the general mean for each constituent.

Before preparing these frequency distributions, the difference necessary for statistical significance was computed for the variance between sides within plants for bacon, and from the variance within plants at different times for the pickles. These necessary differences were then used as the class interval in preparing the frequency distribution for each constituent. This method has the advantage of distributing the observed values over the number of classes that can be distinguished experimentally from one another, although individual results in adjacent classes may not differ significantly. It must be recognized, however, that a large number of classes will be distinguished if the material is variable with respect to the measurement in question, or if the variance between sides and pickles from a given plant is small, and vice versa.

The frequency distributions for the chloride, nitrate, and nitrite contents of the bacon and pickle appear in Fig. 1. Four classes can be distinguished in the bacon with respect to chloride content. Since the distribution around the mean is symmetrical, with only a few plants falling in the extremes, the

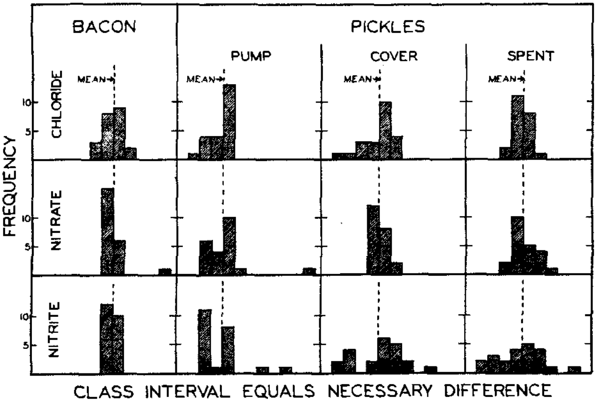

FIG. 1. *Frequency distributions of chloride, nitrate, and nitrite contents of bacon and pickle from different plants.*

chloride content of the product can be considered satisfactory. With the exception of the one plant using a high nitrate content in its pump pickle, only two classes can be distinguished with respect to the nitrate and nitrite contents of the bacon. This is due to the considerable difference observed between the content of these constituents in sides from the same plant.

The chloride content of the pump pickles falls into four classes, the distribution showing that the majority of the pickles are approximately saturated. Since the necessary difference for the pump and cover pickles was approximately the same, the more variable chloride content of the cover pickle is shown by a distribution over six classes. Only four classes of spent pickle could be distinguished.

The nitrate contents of the several pickles fall into three or five classes. Since the necessary differences were relatively large for this constituent, considerable variability is indicated. It has already been shown (Table IV) that the nitrate content of the pickles affects that of the bacon. It appears therefore that the use of pickles of more uniform nitrate content in the different plants would be desirable.

The nitrite content of the pickles was more variable than either the chloride or nitrate contents, since, in spite of the relatively large necessary differences, the values are scattered over about seven distinct classes. Although no direct relation could be demonstrated between the nitrite content of the pickle and bacon (Table VI), and in fact, there was some indication that the properties of the individual side determine its nitrite content, it would nevertheless seem desirable to standardize the nitrite content of the pickles used in the different plants as far as possible. In this connection it must be kept in mind that the combination of nitrite with the muscle pigments and proteins may occur differentially in different sides, and thus contribute to the observed variability.

In conclusion it should be pointed out that, although these results show statistically significant differences between the chloride, nitrate, and nitrite contents of bacon and pickle from different Canadian packing plants, it is not known that these variations seriously affect the final quality of the product.

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CANADIAN WILTSHIRE BACON

III. pH, OXIDATION-REDUCTION POTENTIAL, AND MISCELLANEOUS MEASUREMENTS ON BACON AND PICKLE[1]

By W. H. Cook[2] and A. E. Chadderton[3]

Abstract

The pH of bacon was relatively uniform, but it was possible to demonstrate statistically significant differences between sides cured in the same plants. Sides from different plants were however no more variable than those from the same plant. The results indicate that the pH of the bacon is affected by the pH of the pump pickle, decreases with the time in cure, and increases with the age from cure.

The absolute values of the Eh potentials observed in bacon were doubtful, but since the measurements indicated a statistically significant difference between sides from different plants it appears that this property may be a function of curing practice. Although the moisture content of bacon was relatively uniform, there was a significant difference between sides from different plants, and a significant loss of moisture during maturation and smoking.

The protein content of the tank pickle from different plants varied considerably, and probably reflects the effect of different handling practices. Nevertheless it was possible to demonstrate a direct relation between protein content and pH of the pickle.

Introduction

This paper constitutes one of a series covering an investigation of factory-cured Wiltshire bacon. An outline of the complete investigation and the methods employed were reported in the first paper (7). Of the many factors that may affect the quality of bacon, only a limited number can be controlled in commercial practice. For instance, the salt, nitrate, and nitrite contents of the pickle, and certain curing practices, such as the method of pumping sides, and the curing time, are ordinarily controlled and standardized within a given factory. This gives some control over the composition of the product with respect to the curing salts. These constituents and practices have been dealt with in an earlier paper (8). Other factors which are not controlled to any extent in either the pickle or the product, except indirectly, may also have some influence on the quality of the bacon. These include the pH and oxidation-reduction potential of the pickle and product, the protein content of the pickle, and the moisture content of the bacon. This paper deals with measurements of these properties in bacon and pickle from different packing plants.

Certain evidence (4, 5, 9) indicates that the pH of both the pork and the pickles may affect the final quality of the bacon with reference to its water and salt content, colour, and subsequent taint development. Indirect inform-

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ation suggests that the oxidation-reduction potential may influence the colour. Brooks (2) has shown that, even in the absence of oxygen, the reaction between haemoglobin and nitrite yields a mixture of both nitrosohaemoglobin and methaemoglobin, and that the presence of a reducing agent is required to prevent the formation of the latter compound. Since methaemoglobin is not found to any extent in the interior of bacon, it follows that the tissues contain some reducing substance having an oxidation-reduction potential lower than that of haemoglobin-methaemoglobin (6).

Other results (3) indicate that the moisture content of the lean part of the bacon decreases as the salt content increases, even after due allowance has been made for the salt present. The protein content of the tank pickle may affect other properties, for example, rate of bacterial development and consequently nitrite production, and thus indirectly affect the quality of the final product. The results of these miscellaneous measurements are reported in this paper. The interrelations between the several constituents in, and properties of, the product will be discussed in a later article.

The detailed observations on the several properties were reduced by statistical treatment (12) and are presented in the same form as that used in an earlier paper (8).

Hydrogen Ion Concentration

The mean pH values over all samples of bacon and pickle, and their standard deviations on a single observation basis, appear in Table I. The results show that the pH of the bacon from the various sources was remarkably uniform, with a mean value of pH 5.74. In general, the pump pickles were

TABLE I

pH OF BACON AND PICKLE

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean	5.74	7.86	6.80	6.42
Standard deviation	0.14	0.58	0.67	0.21

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.0064	18	0.291	16	0.270	16	0.048
Between samplings	1	0.1023**						
Between sides within plants	22	0.0264**						
Between plants	21	0.0371	17	0.379	15	0.646*	15	0.040

* *Indicates 5% level of significance in this and subsequent tables.*
** *Indicates 1% level of significance in this and subsequent tables.*

alkaline, having a mean pH of 7.86. The tank pickle was slightly acid initially and became more acidic during cure. Even at the end of cure, however, it was still more alkaline than the sides. The variability in the pH of the pickles appears to be related to their protein content and buffer capacity, since spent pickle, having the highest protein content (Table V), is the least variable.

The results of an analysis of variance appear in the lower part of Table I. In spite of the small over-all variation in the pH of the bacon, the differences between samplings and between sides from the same plant were highly significant, while the differences between sides from different plants were not *significantly greater than those observed within plants.* Other investigations (4, 5, 11) have shown that there is considerable variability in the pH of pork. The findings suggest that the small variations in the pH observed in bacon may be due primarily to differences in the pH of the pork.

The difference between the pH of the pickles from different plants was significantly greater than the variations within plants for the cover pickle only. There was some evidence that there was a significant variation in the pH of the pump pickle in a given plant from time to time, but for reasons already given (7) it was impossible to distinguish precisely between variability originating from this source and that attributable to sampling and other errors.

Simple correlation coefficients were computed between the mean pH of the bacon over all samplings and the pH of the pickles and other known curing practices, as outlined in Table II. The three significant correlation coefficients obtained show that the pH of bacon is inversely associated with the days in cure and with the pH of the tank pickle × days in cure, and directly related to the pH of the pump pickle. It appears that the number of days in cure is primarily responsible for the significance of the combined quantity.

TABLE II

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN THE pH OF PICKLE AND pH OF BACON

Quantities correlated	D.f.	*r*
Mean pH of bacon over all sides and samplings with:		
Pump stitches per side	20	0.00
Days in cure	20	−0.52*
pH of pump pickle (mean)	20	0.46*
pH of tank pickle (mean)	20	−0.09
pH of pump pickle (mean) independent of pH of tank pickle (mean)	19	0.49*
pH of tank pickle (mean) independent of pH of pump pickle (mean)	19	−0.20
pH of pump pickle (mean) independent of days in cure	19	0.32
Days in cure independent of pH of pump pickle (mean)	19	−0.41
pH of pump pickle (mean) × stitches per side	20	0.09
pH of tank pickle (mean) × days in cure	20	−0.50*

Partial correlative coefficients between the pH of the bacon and the pH of the pump pickle independent of days in cure, and days in cure independent of pH of pump pickle, were both insignificant. These results show that although both these factors influence the pH of the bacon, it cannot be said which is the more important.

Certain supplementary studies were therefore made to obtain more definite information. It was found that while the pH of pork was scarcely affected by immersion in pickles at pH 5.0 to 5.5 for periods of a day or two, the injection of relatively small quantities of acidic but unbuffered brines, comparable with pump pickle, had an immediate and permanent effect on the pH of the pork. This suggests that if it is desirable to modify the pH of pork by means of the pickle, acidification of the pump, rather than the tank pickle would be the more effective method.

It is known that the pH of pork varies considerably (4, 5, 11). Unpublished results indicate that under Canadian conditions it may range from pH 5.5 to 6.5. Since the pH of the bacon was 5.7 (Table I), it appears that the meat must have become more acidic during cure. This could scarcely have resulted directly from the injection or absorption of pickle, since the pickles were generally alkaline to pH 5.7. These considerations suggest that the extent of the reaction affecting the pH of the bacon is affected by the length of the curing period independent of the pH of the curing pickles.

Oxidation-reduction Potential

The methods employed for measuring the oxidation-reduction potential of both the bacon and pickle have already been described (7). These results were subject to some uncertainty for the following reasons: (i) the potentials observed in both bacon and pickle showed considerable "drift" with time, and although the readings were not accepted until equilibrium had apparently been established, it is probable that errors of the order of 5 to 10 mv. occurred in some samples; (ii) since the pickle samples were exposed to air during transport, sampling, and measurement, it seems likely that the observed potentials were somewhat higher than those occurring in the curing tanks; (iii) for bacon two apparently identical electrodes gave, on the average, widely different absolute potentials.

In spite of these uncertainties the results are reported in Table III, since certain deductions are possible. The values reported for bacon were obtained with the No. 1 electrode, which gave a mean potential of about 24 mv. as compared with a value of about -121 mv. obtained with the No. 2 electrode on the same samples. The results obtained with the No. 1 electrode are preferred since it reproduced, from time to time, the correct potential in a quinhydrone-buffer solution more closely and consistently than the No. 2 electrode.

All three pickles were found to have about the same mean potential. It seems probable that the observed values represent the actual potentials in

TABLE III

OXIDATION-REDUCTION POTENTIAL (EH) OF BACON AND PICKLE

Statistic	Bacon (Electrode No. 1)	Pickle		
		Pump	Cover	Spent
Mean, mv. (+)	23.7	338	328	328
Standard deviation, mv.	89.9	31.8	30.2	35.6

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	31	2,489	18	975	16	554	16	628
Between samplings	1	9,025						
Between sides within plants	22	1,636						
Between plants	21	22,422**	17	1,044	15	1,204	15	1,952*

the pickles as received, as they were reproducible, within the error attributable to "drift", with different electrodes. Since these samples had suffered exposure to air, however, there is some doubt that they represent material at all comparable with that existing in a curing tank.

In order to obtain information on this point, the oxidation-reduction potential was measured daily at four positions in a tank during cure. The four positions represented two depths, 4 in. and 3 ft. 10 in. below the surface, at two places in the tank. These readings varied considerably with position, depth, and time, and further information is required before a definite statement can be made as to the effect of these variables. It is sufficient to point out that the maximum, minimum, and mean values observed were 245, −189, and 86.2 mv. respectively. Since these values are considerably lower than those reported for tank pickle in Table III, it would appear that a satisfactory estimate of the oxidation-reduction potential can only be obtained on samples that have not suffered undue exposure to air.

Although the observed potentials in the main series of experiments may not be absolute, the results of an analysis of variance are of interest. The difference between plants, for bacon and spent pickles, was the only statistically significant source of variance, distinguishable from experimental error. In this respect both the electrodes used in bacon yielded similar results. It appears from this that the oxidation-reduction potential of bacon is dependent on the handling and curing practices followed in a particular plant.

Because of the uncertainty of the results, only a few correlation coefficients were computed. The coefficient between the Eh of bacon (No. 1 electrode) and that of the tank pickle (mean of cover and spent) was insignificant ($r = 0.25$ for 19 degrees of freedom). Although the Eh of both spent pickle

and bacon differed significantly between plants, they were not significantly correlated ($r = 0.11$).

The results presented in an earlier paper (8) showed that the nitrite content of the bacon was related to the number of stitches used in pumping, independent of the nitrite content of the pump pickle. It was felt that this might be the result of the introduction of air or bacteria with the pump pickle. Since the introduction of air might affect the oxidation-reduction potential of the bacon, the correlation coefficient between this quantity and the number of stitches per side was computed. The insignificant value ($r = 0.03$) was obtained. This does not necessarily disprove the above hypothesis, as the measurements on the bacon were made a considerable time after the pumping operation.

Moisture Content of Bacon

The moisture content of the bacon was determined at all three samplings. The results, including the analysis of variance, appear in Table IV. The mean moisture content over all sides and samplings was 71.38% with a relatively small variation. Nevertheless, an analysis of variance showed that the differences between samplings and between sides from different plants were highly significant. The loss of weight during storage and smoking doubtless accounts for the differences between samplings, a subject to be discussed further in a later section of this paper. The differences between the moisture content of the sides from different plants may be the result of the differences in salt content (3). These relations will be presented in a later paper.

TABLE IV

MOISTURE CONTENT OF BACON

Statistic	
Mean, %	71.38
Standard deviation, %	1.58
Coefficient of variability	2.22

Analysis of variance

Variance attributable to	D.f.	Mean sq.
Sampling and analytical error	86	0.621
Between samplings	2	93.968**
Between sides within plants	22	0.970
Between plants	21	3.100**

Protein Nitrogen Content of Pickle

The results of the Kjeldahl nitrogen determinations appear in Table V. Although expressed as the percentage of protein nitrogen this determination also includes lower compounds which yield ammonia on digestion. The mean protein nitrogen content of the pump pickle was 0.0008%. In some

instances, however, the samples contained almost as much as certain cover pickles. No statistical computations were made on the results obtained with pump pickles. The results in Table V show that the mean protein nitrogen content of cover pickle was about 0.09%, while that for spent pickle was 0.12%. The analysis of variance shows that the protein content of the pickles from different plants differs significantly, and probably reflects the effect of the different handling, treatment, or storage practice.

TABLE V

Protein nitrogen content of pickle

Statistic	Cover	Spent
Mean, %	0.092	0.121
Standard deviation	0.049	0.040
Coefficient of variability	53.5	33.2

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error and variance between samplings	16	0.00032	16	0.00053
Between plants	15	0.00467**	15	0.00278**

Although the observed variations in tank pickle could be accounted for in this way, an attempt was made to determine whether the solubility of the protein was dependent on any other properties of the pickle. The computation of correlation coefficients between the protein content and the pH and salt content of the pickle yielded values of 0.63 (highly significant), and 0.25 (not significant), respectively. It is therefore concluded that, whereas the observed variations in the salt content of the pickle (8) have no effect, the solubility of the proteins increases with increase in pH within the range (Table I) experienced in practice. This latter finding is in general agreement with the results of other investigations (11).

Difference Between Samplings

It has already been pointed out (7) that the design of the experiment did not permit accurate estimation of the effect of time, as the observed difference between samplings included both systematic differences between the positions from which successive samples were taken, and the true effect of ageing. The results obtained with salt and nitrate (8) led to the conclusion that a systematic difference between positions did exist. In consequence the observed differences between the nitrite contents at the several samplings had to be attributed to the effect of position rather than time. The question therefore arises as to whether the observed changes in the pH and moisture content of the bacon

represent a real change with time or merely variation between different positions.

The mean pH and moisture content of the bacon at each sampling is given in Table VI. These results show that both the pH and moisture content decreased significantly between successive samplings. Since evaporation is to be expected during storage and smoking, the decrease in moisture content doubtless indicates a real effect of time rather than the influence of position.

TABLE VI

DIFFERENCES BETWEEN SAMPLINGS IN pH AND MOISTURE CONTENT OF BACON

Constituent or property	Mean by samplings			Remarks
	First	Second	Third	
pH	5.78	5.71	—	Differences between means highly significant
Moisture, %	72.83	71.39	69.91	Differences between any two means highly significant

The decrease in pH between samplings, although significant, is rather small, and might have resulted from the influence of position rather than a real change with time. Earlier results (Table II) indicate that the pH of bacon may be affected by the time in cure, and it is reasonable to believe that the pH might also be affected by the time from cure. The difference between the mean pH values at the different samples is not a satisfactory method of studying the effect of ageing, since the sides varied in age from 2 to 11 days at the time of the first sampling. Consequently, any change that takes place with time might reasonably have occurred to various extents in the sides, quite apart from the uncertainties arising from the effect of position.

In order to obtain more definite information on the effect of time, at various stages, on the pH of bacon, simple and partial correlation coefficients were computed between the pH of the bacon and the elapsed time before, during, and after cure. By making these computations separately for each sampling, the possible effect of differences between the positions from which successive samplings were taken was excluded. The results appear in Table VII.

Since the correlation coefficient between pH and days from slaughter to cure was not significant, it appears that the ordinary variations in cooling time have no effect on the acidity of the smoked bacon.

Since the pH of the pump pickle and the period in cure have been shown to affect the pH of bacon to some extent, it might be that the length of holding period prior to cure had an effect on the pH of pork which was subsequently altered by other factors. However, the results of investigations on rabbit and poultry muscle (1, 10), and some unpublished results on pork, indicate that the ultimate pH of muscle tissue is attained well within the shortest cooling period used in practice.

TABLE VII

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN pH OF BACON AND TIME IN CURE AND AGE FROM CURE

Quantities correlated	D.f.	r
pH of bacon—first sampling with:		
Days from slaughter to cure	15	−0.01
Days in cure	20	−0.53*
Days from cure to first sampling	19	+0.05
pH of bacon—second sampling with:		
Days in cure	19	−0.39
Days from cure to second sampling	19	+0.53*
pH of bacon—first and second samplings with:		
Days from cure when measurement made for both samplings	40	−0.09
pH of bacon—first sampling with:		
Days in cure, independent of days from cure to first sampling	18	−0.51*
Days from cure to first sampling, independent of days in cure	18	0
pH of bacon—second sampling with:		
Days in cure, independent of days from cure to second sampling	18	−0.40
Days from cure to second sampling, independent of days in cure	18	+0.53*

The correlation coefficients between pH and time in cure were negative, and significant at the first, but not at the second sampling. The coefficients between pH and time from cure were positive and significant only for the second sampling. Similar results were obtained when the opposing effects of time in cure and time from cure, were rendered independent by partial correlation. This indicates that the pH of bacon decreases during cure but increases during maturation.

The increase in the pH of bacon during maturation was confirmed by the results of a supplementary experiment in which ground samples of bacon were stored at 1 to 2° C. for some time. The final pH values attained varied from 6.0 to 8.3. It was also observed that the colour and final pH of the bacon appeared to be related. All samples at about pH 6.0 were grey, those at pH 7.0 greyish-brown to brown, while those at pH 8.0 or higher had retained their red colour. A further investigation of the effect of pH on colour change has been projected.

Difference Between Bacon and Pickle from Different Plants

In a previous paper (8) dealing with the chloride, nitrate, and nitrite contents of bacon and pickle, it was found that the differences between plants were usually the major source of variation. The properties and constituents considered in the present paper did not show such marked variation between plants. With respect to pH, significant differences between plants could be demonstrated for cover pickle, but not for the pump or spent pickle, or for the bacon. The oxidation-reduction potential measurements showed significant differences between the bacon and spent pickles from different plants.

The different practices followed in the different establishments were reflected by highly significant differences in the moisture content of the bacon, and the protein content of the cover and spent pickles.

The generalizations summarized in the above paragraph are evident from the results presented in earlier tables. Since individual plants seldom differed greatly from the general means, and since the factors considered in this paper could only be controlled indirectly, more detailed discussion of this source of variation is considered unnecessary.

Acknowledgments

The authors wish to express their appreciation of the assistance and co-operation of the individuals and firms referred to in the first paper, and in particular to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on statistical matters.

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CANADIAN WILTSHIRE BACON

IV. CORRELATION BETWEEN CONSTITUENTS AND PROPERTIES OF BACON[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

Simple correlation coefficients computed between several observed properties and constituents of factory-cured Wiltshire bacon showed the following to be associated: nitrate and chloride contents; nitrite and chloride contents; nitrite content and pH; and moisture and chloride contents. The first two of these associations probably arise from the pickle compositions and curing practices followed in the plants. The last two associations suggest a certain degree of dependence between the two properties, i.e., the nitrite content of the meat increases with the pH, and the moisture content decreases as the chloride content increases. When the moisture content of the bacon was expressed on a salt-free basis, the correlation between the moisture and chloride contents was not significant. This indicates that curing practices favouring a high salt content do not result in the removal of more moisture from the sides.

Introduction

The chloride, nitrate, nitrite, and moisture contents, pH values, and oxidation-reduction potential of factory-cured Wiltshire bacon and the pickles used for its manufacture, have been reported in earlier papers of this series (3, 4). These papers also reported the degree of correlation between similar constituents in the pickle and product. This paper deals with the correlations between the measured properties of the bacon.

Procedure

The methods employed for making these measurements have already been described (5, 8). The computation of correlation coefficients (7) between the observed quantities over 44 sides, representing 2 sides from each of 22 packing plants, served to determine whether statistically significant correlations existed between the measurements. It must be recognized, however, that a significant correlation between two quantities merely demonstrates that they are associated, and does not necessarily indicate that one is dependent on the other. Even where it seemed reasonably certain that one quantity was dependent on another, it was frequently difficult to determine which of the two was the causal agent. Thus a highly significant negative correlation between the pH and salt content of bacon could indicate either that the salt penetration was more rapid in the more acid sides, or that the pH decreases as the salt content increases.

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Results

Simple Correlation Coefficients

The simple correlation coefficients between these quantities appear in Table I. These coefficients were computed from the results obtained for each sampling at which the measurements were made, and also between the mean values for each constituent over all samplings for each of the sides analysed. This procedure was followed because certain of the observed differences between samplings were attributable to the differences between positions (4, 5) from which successive samples were taken, while others may have represented a real change with time. In consequence it seemed desirable to compute the correlation coefficients from the results obtained at each sampling independently. On the other hand, the variations between different sides for certain properties were small. In such instances it was felt that the variance attributable to over-all sampling and experimental error for any two properties being studied might be sufficiently large to mask any existing correlation. Consequently it was desirable to use in addition the mean values for each measurement over all samplings in order to reduce the variance attributable to sampling and analytical error.

TABLE I

SIMPLE CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 42, for all except bracketted value = 30. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling			
	First	Second	Third	Mean of all
Nitrate in bacon with:				
Chloride in bacon	0.30*	−0.26	—	0.40*
Nitrite in bacon with:				
Chloride in bacon	0.45**	0.28	—	0.38*
Nitrate in bacon	−0.05	−0.23	−0.04	−0.07
Moisture in bacon	−0.04	−0.04	−0.06	−0.02
pH of bacon	0.56**	0.30*	—	0.47**
Eh potential of bacon (El. 1)	—	0.13†	—	0.16
Eh potential of bacon (El. 2)	[0.10]†	0.37*†	—	—
Moisture in bacon with:				
Chloride in bacon	−0.56**	−0.46**	—	−0.60**
Nitrate in bacon	−0.48**	−0.06	−0.07	−0.25
pH of bacon	0.18	0.17	—	0.13
Eh potential of bacon (El. 1)	—	0.05	—	—
pH of bacon with:				
Chloride in bacon	0.09	−0.23	—	−0.04
Nitrate in bacon	0.04	−0.05	—	—
Eh potential of bacon (El. 1)	—	—	—	0.26

† *Correlated with mean nitrite content over all samplings.*
* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

It is evident that values for a number of possible correlation coefficients do not appear in Table I. Most of these omissions result from the fact that several of the determinations were not made at the third sampling. Since the observed oxidation-reduction potentials of the bacon are subject to some uncertainty for reasons already given (3), only a few computations relating this quantity to other properties were made.

The majority of the correlation coefficients reported in Table I are not significant and do not require comment. Significant positive correlations were obtained between the chloride and nitrate contents of the bacon at the first sampling and between the means over all samplings. These coefficients doubtless reflect the association between the concentration of these two substances in the curing pickles or between the curing practices used in different plants, and are therefore of little consequence.

A significant positive correlation was obtained between the nitrite and chloride contents at the first sampling, and between the mean nitrite and chloride contents over all samplings. There was also a significant positive correlation between the nitrite content and the pH of the bacon at all samplings.

Significant negative correlations were obtained between the moisture and chloride contents at all samplings, and between the moisture and nitrate contents at the first sampling. The only significant correlation involving the oxidation-reduction potential was obtained with nitrite at the second sampling, the No. 2 electrode being used. Since these measurements were uncertain, and those with Electrode 2 probably less reliable than those with Electrode 1 (3), further comment is unnecessary.

Partial Correlation Coefficients

The relation between the nitrite content and the pH and chloride content, and that between the moisture content and the chloride and nitrate contents, were investigated further by computing partial correlation coefficients between these quantities. The values obtained appear in Table II. The correlation coefficients between the nitrite and chloride contents, independent of pH, were positive and highly significant at both the first and second samplings independently, and also between the mean values for each side over all samplings. There is therefore little doubt that the nitrite content of these sides increased with their chloride content. It seems probable, however, that these two constituents are not dependent on one another but are merely associated through the pickle compositions or curing practices followed in the different plants.

The coefficients representing the degree of correlation between the nitrite content and pH, independent of chloride content, also indicate a definite association between these quantities, the nitrite content increasing as the pH increases. Certain evidence indicates that the pH of pork (2, 6) and the nitrite content of the bacon (4) are properties of the individual side and to that extent are independent of the curing practices followed. Consideration of these facts sug-

TABLE II

Partial correlation coefficients between observed constituents and properties of bacon

(Degrees of freedom = 41. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling		
	First	Second	Mean
Nitrite in bacon with chloride in bacon independent of pH of bacon	0.48**	0.59**	0.45**
Nitrite in bacon with pH of bacon independent of chloride in bacon	0.59**	0.39**	0.53**
Moisture in bacon with chloride in bacon independent of nitrate in bacon	−0.50**	−0.49**	−0.56**
Moisture in bacon with nitrate in bacon independent of chloride in bacon	−0.39**	−0.21	−0.01

** *Indicates 1% level of significance.*

gests that there may be a dependence of one of the properties on the other rather than a mere association. If this is so it seems likely that the nitrite content is dependent on the pH rather than the reverse, since it is highly improbable that the small quantities of nitrite present would have any effect on the pH of the bacon. Such a dependence could be explained in several ways, namely: a more rapid penetration of the nitrite from the tank pickle as the pH of the meat increases; a more rapid production of nitrite from nitrate at higher pH levels; or a decreased rate of combination of nitrite with the muscle proteins and pigments at high pH levels. It has been shown that a high electrical resistance is associated with a high pH, and a slow penetration of chloride (2). If the absorption of nitrites is comparable with that of chlorides it seems unlikely that the first explanation is adequate for the observed differences in pH. Likewise it would appear that the rate of combination of nitrite with the muscle pigments would not be affected appreciably (1) by the variations in pH at the levels observed (3) in these sides. On the other hand the rate of reduction of nitrates by bacterial activity might reasonably proceed more rapidly as the pH increased within the observed range.

The results in Table II show that the correlations between moisture and chloride contents of the bacon, independent of nitrate content, were highly significant at each sampling independently, and for the mean values over all samplings. These show quite definitely that the moisture content decreases as the chloride content decreases. The correlation between the moisture and nitrate contents, independent of chloride content, was significant only for the results of the first sampling. It appears therefore that although the nitrate content may have a slight independent effect on the moisture content of the bacon, it is of secondary importance compared with that of chloride.

Relation between Moisture and Chloride Contents

The decrease in the moisture content with increase in chloride content of the whole bacon may merely reflect the effect of the additional dry matter present as sodium chloride. On the other hand it may be due to this effect plus the influence of certain curing practices, if conditions favouring the absorption of greater quantities of chloride also favour the loss of additional quantities of moisture from the sides. In order to eliminate the direct effect of the chloride on the dry matter content, the moisture contents, as previously reported (3), were computed on a sodium chloride-free basis for each side. When expressed as such, the mean moisture content was found to be 74.03% with a standard deviation of 0.70%, as compared with a mean of 71.38% and a standard deviation of 1.58% (3) calculated on the basis of the whole bacon. It is therefore evident that the direct effect of variations in the chloride content was a major source of variation in the moisture content of different sides.

The correlation between moisture, on a salt-free basis, and sodium chloride content was not significant ($r = 0.23$, for 40 degrees of freedom). The negative relation between the chloride and moisture contents of whole bacon, noted previously, was therefore due entirely to the additional dry matter contributed by the sodium chloride. These findings, together with the fact that the moisture content of bacon on a sodium chloride-free basis was essentially the same as that of fresh pork, indicate that the net result of pumping and curing is to increase the salt content, with little, if any, loss of moisture.

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ON THE LIFE CYCLE AND OTHER ASPECTS OF THE SNAIL, *CAMPELOMA*, IN THE SPEED RIVER[1]

BY J. C. MEDCOF[2]

Abstract

Judged from a statistical study, the life span of the snail is five years. Parturition occurs in summer and year classes are recognizable. Sizes of approximately 12, 17, and 23 mm. are reached in one, two, and three years respectively. Sexual maturity is reached in two years and reproduction is parthenogenetic. Some snails hibernate and some aestivate. Rest marks on shells and opercula and a limy deposit in the protoconch were studied.

In 1934 the writer's attention was directed by Mr. J. G. Oughton of the Royal Ontario Museum of Zoology, Toronto, to a colony of a single kind of freshwater snail, *Campeloma*, living in the Speed River at Hespeler, Ontario. The specific identification of the form is undecided because no careful taxonomic study of the genus has been made yet. Mr. F. C. Baker of Urbana, Illinois, has examined collections and states that the form is allied to *C. decisum* but that it is probably a new species.

Successive random samples of the snails were collected from the river on July 9, 1934, November 11, 1934, February 3, 1935, and March 8, 1936, and the shell heights measured to the nearest millimetre with calipers. A separate record was kept of the measurements of empty shells occurring in the samples except in 1935, when they were not made. The results of the measurements are presented as size-frequency distribution curves, plotted on a percentage basis, in Fig. 1. Only two curves have been prepared from the measurements of the empty shells—one for the two 1934 samples combined and the other for the 1936 measurements alone.

In their study of *C. rufum* in the Salt Fork River, Illinois, Van Cleave and Altringer (7) have justified the treatment of clear modal groups such as appear in the present curves as year classes. The present data are considered in the same way and summarized in Table I. Three maxima are usually apparent in each curve.

GROWTH RATE

Table I was used as a basis for constructing the growth curve, Fig. 2, by merging the data for the separate year classes represented. There seems to have been little variation in the growing conditions during 1934 and 1935,

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so this procedure seems justified. Although the largest reported Salt Fork River shells measured 40 mm. and were considered three years old, the writer has found Speed River shells as large as 47 mm. It seems improbable from the growth curve that this size could be attained in the Speed River in less

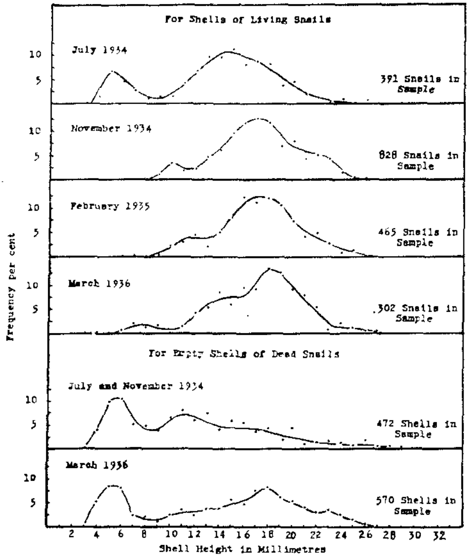

FIG. 1. *Size-frequency distribution of heights of snail shells.*

TABLE I

SHOWING MODAL VALUES TAKEN FROM FIG. 1

Date of collection	First maximum, mm.	Second maximum, mm.	Third maximum, mm.	Fourth maximum, mm.
July 1934	6	15	19	—
Nov. 1934	10	17	23	—
Feb. 1935	12	17	—	—
Mar. 1936	13	17	—	—
Empty Shells				
1934 collections	6	12	18	—
1936 collection	5	11	18	23

than five years. The northern form, then, grows more slowly, lives longer, and reaches a greater ultimate size than the Salt Fork River snail.

Winter Conditions

Fig. 2 shows that in the Speed River colony there is no slackening in the growth rate during the first winter, but that in the second there is a marked depression. The reason for this pause may be that at this time the two-year-old snails are developing their first brood of uterine young. Reference to the frequency curves for empty shells (Fig. 1) shows evidence suggesting that the winters are particularly hard on the snails. A high mortality among the newly born is indicated by the maxima at 5 and 6 mm. The writer has not attributed this to any particular cause. The other three modal values, however, at 11, 18, and 23 mm., if marked on the mean growth curve (Fig. 2) will each be found to have a corresponding "winter" position on the abscissal time scale. Winter killing is probably largely responsible for the multimodal nature of the frequency curves for empty shells.

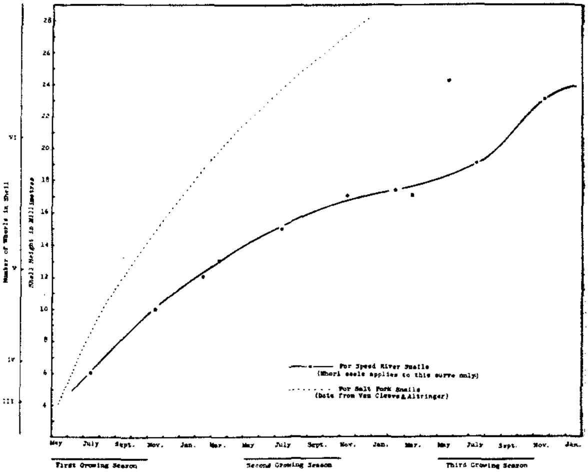

Fig. 2. *Growth rate of shells.*

Call (2) has said that in the fall of the year the snails *(Campeloma)* bury themselves deeply in the mud and hibernate until spring. Van Cleave and Altringer found that the Salt Fork River stock remained active all winter long. This apparent disagreement illustrates the flexibility of the animal's

behaviour, because the present study corroborates both observations. In the stream it was found that throughout the winter the snails continued to crawl about over the hard bottom and could be collected easily by clearing away the ice. On the same days *Campeloma* in the pond were found to be buried to some depth in the bottom, as will be described.

Above the point in the river where the sampling was done there is a mill pond, a hundred acres or more in extent, held back by a dam. The pond bottom here is of soft black muck often smelling strongly of hydrogen sulphide. Throughout the warmer parts of the year there is a congregation of snails (*Campeloma*) on the bottom beginning at the pond margin and extending out to a depth of 10 or 12 in. Here the snails are usually so plentiful that it is seldom more than an hour's work to collect 200 or 300 of them. (The reason for this summer congregation is obscure. It may be related to oxygen depletion of the water at greater depths owing to decomposition of the bottom muck.) Strangely enough, by November 10, 1935, when the surface water temperature was 6.8° C., these snails had completely disappeared from the margin of the pond but were found at greater depths by dredging from a boat. During the winter they were secured by dredging mud through a hole in the ice. After some exposure to air a number of the latter extruded their young.

On March 24, 1935, the writer was fortunate to be at the pond during the period of emergence from hibernation. The surface water temperature was 6.6° C., the ice had "gone out" on March 17, and March 22, 23, and 24 were all bright warm days. On this day there were very few snails to be found about the pond margin, but several were collected from 2 or 3 ft. of water. Many of these were crawling towards shore. In each case the pubescent covering of the shell and the operculum were impregnated with an adhesive layer of fine silt. Besides this the periostracum and even the pearly layers of the shell itself were dyed by the black muck. This dark colour persisted even after thorough washing and scrubbing and after months of preservation. None of these peculiarities are to be found in shells taken from the pond at other times of the year and never in those taken from the river itself. The shells must be bleached out to their normal greenish colour during the spring, for by July they resemble the river shells in every detail. It seems reasonable to attribute the change to hibernation burial in the muck of the pond bottom.

Aestivation

Aquatic pulmonates are known to practise aestivation and to form epiphragms in the mouths of their shells as do their terrestrial relatives (3). Aestivation is much less common among the larger branchiate types. Mr. William LeRay of the University of Toronto recounts that in August 1927 he found *Campeloma* in a vigorous condition along with crayfish under stones in the dry bed of a brook that normally flows into the Crane River near Johnston's Harbour, Ont. It is unusual to find *Campeloma* except in permanent waters, but it would seem that it can survive in temporary brooks for some time.

Reaction to Change in Water Level

An example of striking contrast in behaviour of the snails in different habitats may be cited. Rapid changes in the water level of the Speed River pond are frequently brought about by the varying needs of the mill and by other factors. These changes affect the pond snails during the summer, for they were observed to travel back and forth maintaining a position just at the water's edge. In some cases they were observed to travel as much as 12 ft. overnight in places where the beach sloped gently.

The opposite extreme was found among snails living on the flat surfaces of rocks below the dam. Here the stones were completely overgrown with a dense mat of *Cladophora* except for small circular patches which were covered by the extended feet of snails. The shells of these animals themselves bore a heavy growth of *Cladophora* and it appeared that they remained permanently attached to the small bare patches of rock. On one occasion marked animals were observed over a period of two days throughout which they did not leave their positions.

Parthenogenesis

No male of the species was found during this study, although the genitalia of approximately 450 snails were examined. Mr. J. G. Oughton has made a similar report on the results of his own earlier study of the Speed River snails. Thus it appears that parthenogenesis is the normal form of reproduction in the Speed River colony as in the Salt Fork River stock (5, 7).

Sexual Maturity and Reproductive Capacity

The smallest pregnant snail found during the investigation measured 17.5 mm. in height, contained one embryo, and was collected in February 1935. From Fig. 2 it would appear that, at the earliest, reproduction begins either during the latter part of the second summer or during the second winter. Probably in most cases it is delayed until the third summer, when the length is 19 mm. It is at about this same size or slightly larger that the Salt Fork River snails begin to reproduce, but they are then only one year old. The northern form seems to have a delayed sexual maturation.

TABLE II

THE RELATION BETWEEN SIZE, AGE, AND REPRODUCTIVE ACTIVITY OF 55 SNAILS COLLECTED IN FEBRUARY AND MARCH 1935

Age of snail, years	Size of snail, mm.	No. of this class pregnant, %	Average number of eggs and/or embryos in pregnant uteri
1	5 – 12	0	0
2	13 – 18	18 (high)	2
3	19 – 23	77	6
4	24 – 28	93	15

Table II summarizes the results of uterine examinations made on snails collected early in the spring before parturition began. There is a clear, directly proportional relation between the size of the parent and her reproductive capacity. The highest fertility record, 22 mature and 4 developing embryos, was that of a 28 mm. snail taken from the mill pond on March 24, 1935. A study of Table II with reference to Fig. 1 will demonstrate that the maintenance of the colony depends almost wholly on the reproductive activity of the three-year-olds. The stock of *C. rufum* in Illinois, as shown by Van Cleave and Altringer, is more prolific and probably maintains itself chiefly through reproduction by the two-year-olds.

Table III shows the results of uterine examinations made on snails collected at different times of the year. From this it seems that ovulation and embryonic development go on at all seasons but that parturition is intermittent and occurs only in the summer. In other words, the mature embryos accumulate in the uterus during the winter. These conclusions are much the same as those of Van Cleave and Altringer.

TABLE III

THE RESULTS OF UTERINE EXAMINATIONS OF 73 SNAILS 20 MM. OR MORE IN HEIGHT

Date of collection	Number examined	Empty, %	With eggs or very small embryos, %	With large embryos, %
July 1934	21	33	48	24
Nov. 1934	15	20	60	67
Feb. and Mar. 1934	37	11	78	89

Rest Periods in Shell Deposition

Careful measurements of the height, width, and number of whorls of shells and the heights and widths of the corresponding opercula have shown that there is a precise relation among all these. It has therefore been possible to supply two size scales to the growth curve (Fig. 2), one showing the shell height and the second showing the number of whorls.

Practically all snails more than one year old show at least one heavy brown line paralleling the growth lines somewhere on their shells. The opercula too, ordinarily show one or more concentric rings that stand out more clearly than the growth rings. A study involving the measurements of the relative positions of these heavier lines on the shells and opercula has convinced the writer that the two are formed simultaneously.

The peculiar conditions that favour the formation of these lines have not been discovered. However, a number of specimens that failed to grow appreciably while in a laboratory aquarium for eleven months invariably showed a broad dark brown band at the edge of the shell. It seems likely that line-formation is associated in nature with rest periods in shell deposition, but the occasion for these periods is still in doubt.

Shell Erosion and Structure of the Spire Tip

A large shell dropped into weak acid begins "gassing" at the protoconch immediately but only there, showing that in this oldest region of the shell the limy part is least protected by the periostracum. Shell erosion in the Speed River colony was conspicuously absent, probably because the stream flows through country where the main outcrop is limestone. In some habitats, on the contrary, several of the uppermost whorls may be completely missing from old shells, which may be scarred additionally with deep furrows or pits scattered over other parts.

The writer was curious to know if this removal of the upper whorls inconvenienced the snail and how repairs were made to the shell when it was perforated by erosion. To study this, median vertical sections were made of several shells. It was discovered that although the newly-born snail occupied all the whorls of its shell, it very soon began to fill in the smallest ones with a solid limy deposit. This process appeared to be continuous, a total of 0.7, 1.7, 2.4, and 3.0 whorls being completely filled in by the time the snail reached 10, 20, 30, and 40 mm. in height respectively. The fact that the snail normally abandons the cavity of the protoconch suggests that erosion of the solid part of the spire would not interfere seriously with the life processes.

A microscopic study of thin sections of the shell showed this central secretion to be structurally distinct from the three typical layers. The new deposit is probably secreted by that part of the mantle covering the visceral hump, while the periostracum, ostracum, and hypostracum seem to be formed at the mantle margin (6). With this probable difference in origin it might be expected that the structure would be different. It seems likely that a secretion of a similar type would be useful in making repairs to the shell when it is perforated by erosion.

Reversal of Shell Symmetry

Reversed shells are not infrequently met with in this genus but in the Speed River stock they are rare. Of 530 uterine embryos examined 2 were sinistral, and in a sample of 870 snails above the age of one year, only 2 were sinistral. This is low compared with the Salt Fork River variety (4).

Enemies of *Campeloma*

Baker (1) records several fish, amphibian, reptilian, and bird enemies of *Campeloma*. The writer has no direct evidence of fish preying upon the snail in the Speed River, but he has examined only five stomachs, from two common suckers, two bullheads, and one dace, all large enough to take snails. The writer examined the stomachs of nine bullfrogs *(Rana catesbiana)* from the pond and found that while tadpoles and small frogs constituted a greater part of the diet, snails *(Campeloma)* of various sizes formed an estimated 24% of the bulk of the stomach contents.

Only one observation was made on birds as possible enemies and this was not in the Speed River but near Burnt River at Coboconk, Ont., June 9, 1934.

Freshly regurgitated pellets of the European starling were found at the base of a dead pine tree where the birds were nesting in what had been the nest of a flicker. The pellets contained three snails *(Campeloma)* measuring 20, 22, and 26 mm. in height. None of these had been digested, for the bodies and opercula were still intact within the shells.

A Mite in the Mantle Cavity

Living in the mantle cavity of the snail was a mite, kindly identified by Miss Ruth Marshall as *Unionicola campelomaicola*. In the river proper, 18% of the snails were infested but rarely was there more than one mite in a snail. In the pond stock there was a 46% infestation with an average incidence of 1.6 mites per infested snail.

Besides the above, an insect, *Mesovelia bisignata* (Uhler), identified by Dr. J. D. Detwiler of the University of Western Ontario and regarded as a visitant, was found in the mantle cavity of one snail. In another, one specimen of the predatory leech *Helobdella nepheloidea* (Graf), identified by Dr. J. P. Moore of the University of Pennsylvania, was found.

A Fluke in the Uterus

In the uteri examined during the embryo counts many metacercaria were discovered. There were more in snails collected during the summer than at other times of the year and more in the snails living in the pond than in those taken from the river. The highest infestation was recorded for July 1934, when it reached 88% with an average incidence of 29 flukes per infested animal. The smallest snail dissected in this study was 15 mm. high and it contained five flukes.

Acknowledgments

The writer is indebted to the University of Western Ontario for the provision of the necessary facilities for the conduction of this study; to Professor A. D. Robertson, Head of the Department of Zoology of the University, for the direction of the work during the two years it was carried on; and to Mr. J. G. Oughton of the Royal Ontario Museum of Zoology, for assistance and advice from time to time.

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STUDIES ON *STRONGYLOIDES AGOUTII* SP. NOV. FROM THE AGOUTI (*DASYPROCTA AGOUTI*)[1]

BY HENRY J. GRIFFITHS[2]

Abstract

A morphological and biological study is presented of a hitherto unrecorded member of the genus *Strongyloides* from the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The name *Strongyloides agoutii* sp. nov. is proposed for this species.

Observations on the free-living development over a period of three years showed the indirect type to prevail; no seasonal variation was observed. Continuous propagation of the free-living generation of this species was not observed in faecal cultures or on artificial media.

A brief résumé of the classical studies on species of the genus *Strongyloides* is included, together with a summary of existing hypotheses and theories on the biology of this group. A list of species and hosts for this genus is given.

Introduction

As a general rule, a bisexual parasitic generation alternating with a larval stage outside the host may be considered as characteristic of the mammalian nematode parasites. This larval stage is essential and is subject to many modifications depending upon the adaptations of the parasite concerned. The non-parasitic phase of the life cycle of *Strongyloides* is similar to that of many other intestinal nematodes in that the offspring of the parasitic generation may develop directly into forms infective to a new host. In addition to this, however, *Strongyloides* may produce a true free-living generation consisting of adult rhabditiform males and females, which in turn reproduce sexually and give rise to filariform larvae infective to a new host.

The occurrence of this true free-living generation is uncommon among nematode parasites, and the genera *Strongyloides* and *Rhabdias*, in which it is found, are differentiated from other nematodes of vertebrates by this biologically significant mode of development. Another distinguishing feature of these two genera is the fact that the parasitic stage in the intestine of the host is represented only by female worms, considered by some investigators to be parthenogenetic and by others hermaphroditic. For many years the validity of this fact was unquestioned until Kreis (23) and Faust (9) reported the occurrence of parasitic males in infections of *S. stercoralis* in man and dogs.

The genus *Strongyloides* presents excellent opportunity for detailed study of both the parasitic and free-living forms of nematodes, and offers problems in nematode biology of an intricate and complex nature.

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The studies on the genus *Strongyloides* presented in this paper were undertaken to throw further light on certain inconsistencies recorded in its life history. The free-living and parasitic phases of a hitherto unrecorded strain of *Strongyloides* were observed. The results were compared with those obtained by workers investigating other species.

Materials and Methods

The supply of *Strongyloides* material was provided by the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The animals were kept indoors in wooden frame cages covered with light wire fencing. Each animal was supplied with a sleeping box and bedding of wood shavings, about 2 in. deep. The cage floor was of asphalt, which permitted easy washing and disinfection. The maintenance ration consisted of bananas and coarse rolled oats supplemented by sprouted grain, peanuts, carrots, sweet potato, or apple when available. A small amount of cod liver oil was mixed occasionally with the rolled oats as a source of vitamins A and D.

The room in which the agouti were housed was held throughout the year at a temperature of about 70° F. As the floor of the pens was covered with shavings and as the bedding was usually dry, any possibility of the propagation of various species of free-living nematodes, the introduction of which might have caused considerable confusion, was eliminated.

Owing to the rapid development of the free-living phases of *Strongyloides*, it is very desirable that faeces for culturing be collected and used as soon after passage as possible. The agouti was observed to defaecate at a fairly regular period after feeding and usually in the same location in the cage, so that collection of fresh faeces was a minor problem. When in captivity and fed on the diet detailed above, the agouti passes stools that are moist and solid.

The customary Petri dish, 100 by 15 mm., was found satisfactory for the routine faecal culture observations of the free-living phase of development. On account of the heaviness of infection of the agouti, it was not necessary to culture large quantities of faeces. If too much was used, difficulty was experienced in making accurate observations under the binocular dissecting microscope because of the density of the culture. Cultures were mixed to a semi-solid consistency and kept adequately moistened. During the early stages of observation the cultures were occasionally stirred if a fungus growth appeared. For certain observations the faecal material was mixed with approximately one-half its volume of animal charcoal.

All cultures, unless otherwise stated, were incubated at room temperature, usually 22° to 25° C., but with a possible range of from 15° to 30° C. To prevent undue drying of the cultures during incubation above 25° C., the Petri dish lids were lined with filter paper, which was readily moistened. At temperatures over 30° C., it was more satisfactory to place the cultures over water in large covered culture dishes.

The free-living and parasitic forms of *Strongyloides* were killed and fixed by various methods, and although much care and attention were given to methods employed, considerable shrinkage and distortion of the parasitic forms occurred. Specimens were killed and fixed in hot 70% alcohol containing 3 to 5% glycerine. The container was loosely covered to exclude dust and set aside until the alcohol had evaporated, thus leaving the worms in dilute glycerine, suitable for microscopic examination. This method was found to be quite satisfactory. Hot 70% alcohol was not found to be as satisfactory a fixative as the alcohol and glycerine. Most satisfactory results were obtained by killing the specimens in hot water, fixing in 5% formol-saline, washing, and gradually transferring to glycerine alcohol. Material may be stored in 70% alcohol containing 3 to 5% glycerine for a considerable time; the presence of glycerine is a safety measure against desiccation owing to the evaporation of the alcohol. The ova of *Strongyloides* can be preserved in 5% formol-saline for several months without shrinkage or appreciable distortion.

Remarks on the Genus *Strongyloides*

The homogenicity and simplicity in structure of the members of the genus *Strongyloides* may be considered responsible for the difficulties that have arisen concerning the systematics of this group. Species have been recorded from a wide variety of unrelated hosts, as shown by the following list:

List of Known Species of the Genus *Strongyloides* Grassi, 1879

Type species: *Strongyloides stercoralis* (Bavay, 1876), from man.
Other species:

- *S. akbari* Mirza and Narayan, 1935, from *Crocidura coerula*—India.
- *S. avium* Cram, 1929, from domestic fowl—United States.
- *S. canis* Brumpt, 1922, from dog—Japan and China.
- *S. cebus* Darling, 1911, from *Cebus hypoleucus*—United States.
- *S. chapini* Sandground, 1925, from *Hydrochoerus hydrochoera*—United States.
- *S. felis* Chandler, 1925, from cat—India.
- *S. fülleborni* von Linstow, 1905, from *Anthropopithecus troglodytes* and *Cynocephalus babuin*—Africa.
- *S. longus bovis* de Gaspari, 1912, from ox—Turin.
- *S. minimum* Travassos, 1930, from *Dafila bahamensis*—Brazil.
- *S. mustelorum* Cameron & Parnell, 1933, from *Mustela ermina*—Scotland.
- *S. myopotami* Artigas & Pacheco, 1933, from *Myopotamus coipus*.
- *S. nasua* Darling, 1911, from *Nasua narica panamensis*—Panama.
- *S. ophidae* Pereira, 1929, from *Drimobius bifossatus*.
- *S. oswaldoi* Travassos, 1930, from domestic fowl—Brazil.
- *S. ovocinctus* Ransom, 1911, from *Antilocapra americanus*—United States.
- *S. papillosus* (Wedl., 1856) Ransom, 1911, from sheep, goats, rabbits, etc.
- *S. pereirai* Travassos, 1932, from *Elosia rustica*—Brazil.
- *S. ransomi* Schwartz & Alicata, 1930, from pig—United States.

S. ratti Sandground, 1925, from *Rattus norvegicus*—United States.

S. simiae Hung and Hoeppli, 1923, from *Macaca* sp.

S. stercoralis var. *eryxi* Mirza & Narayan, 1935, from *Eryx johnii*—India.

S. stercoralis var. *vulpi* Mirza & Narayan, 1935, from *Vulpex alopex*—India.

S. suis (Lutz, 1894) von Linstow, 1905, from pig.

S. venezuelensis Brumpt, 1934, from *Rattus norvegicus*—Venezuela.

S. vituli Brumpt, 1921, from ox—France.

S. viviparous (Probstmayer, 1865) von Linstow, 1905, from horse—Europe.

S. westeri Ihle, 1917, from horse—Holland.

In addition the following undetermined species of *Strongyloides* are listed by Sandground (28):

Strongyloides sp. from lemur, Weinberg & Romanovitch, 1908.

Strongyloides sp. from fox, Romanovic, 1914.

Strongyloides sp. from guinea pig, Krediet, 1921.

Hall (20) makes reference to *Strongyloides* sp. from a rodent, but he has been unable to locate any reference to a paper by Parona whereby the observation might be confirmed.

There has been much difference of opinion as to the validity of many of these species. The difficulty of observing and recording any distinct characters on which specific distinction may be made, has led to a tendency on the part of many workers to erect new species on the grounds of the occurrence of the parasite in a previously unrecorded host. In other cases, the description of new species has been based almost entirely on dimensions of the body. These distinctions, probably legitimate in many cases, have seldom been accompanied by drawings that would be of benefit to subsequent investigators. Until recent years, there has been a tendency to neglect the bisexual forms when describing new species. In many species, the free-living forms are remarkably similar in appearance and do not possess distinctive features by which they may be characterized. However, differences do exist in the morphology of the bisexual generation, and in at least one species, *S. fülleborni*, these characters are sufficient for species identification. Goodey (15) has shown that constant differences of structure do exist between the free-living stages of *S. fülleborni* and *S. stercoralis* and that the free-living male of *S. ratti* materially differs from the free males of the other two species mentioned. These observations have been made only by examination of the species in question in as great detail as possible, together with careful camera lucida drawings. Looss (26, p. 215), who has furnished the best and most detailed drawings of *S. stercoralis*, writing on the occurrence of the genus in animals states: "Besides being found in man, species of *Strongyloides* are very frequent in animals (mammals, birds and reptiles); so far as my personal observation goes, they so greatly resemble *Strongyloides stercoralis* that it is difficult to say whether they are the same species or not. The free-living generations on the contrary, so far as I am acquainted with them, show

slight but distinct differences from one another, which make it probable that different species exist."

Chandler (4) has undertaken the comparison of representatives of the genus from monkeys, cats, and man with the published characters of other forms available at that time. He suggests that the forms of *Strongyloides* considered in his paper be grouped into two species, *S. papillosus* and *S. stercoralis*, the species *ovocinctus*, *fülleborni*, *suis*, *simiae*, and *cebus* being reduced to hostal varieties or sub-species of *papillosus*, and the species *nasua*, *canis*, and *felis* to varieties or sub-species of *S. stercoralis*.

It would appear then, that the validity of many species of *Strongyloides* is questionable. Species differentiation has in many cases been based on proportionate lengths of different parts of the body without morphological distinctions. The observations of many investigators show that there may be a very wide range of variation in these ratios even between individuals in the same host, and there is little doubt that unless accompanied by other significant morphological features, they are of no value for specific diagnosis.

At the present time, however, it would seem that lack of data prevents a systematic reorganization of the genus on a morphological, physiological, or biological basis.

Strongyloides agoutii sp. nov.

The creation of numerous species of *Strongyloides* may be unwarranted, yet, when the basis for the taxonomy of this group is eventually laid, such species can be rendered invalid with greater ease and fewer complications than if they had originally been grouped under one specific name. On these grounds, it is considered advisable to create a new species for the strain of *Strongyloides* found in the agouti (*Dasyprocta agouti*).

A summary of the more important dimensions of 25 specimens of the parasitic and free-living generation is given in Table I. All measurements were made on unpreserved material. Specimens were examined in water and were rendered motionless by the careful application of heat.

Parasitic Generation

Female (Fig. 1). The length varies from 3.94 to 6.45 mm., and the width from 0.03 to 0.059 mm., measured at the middle of the body. The body is long and filiform, showing equal thickness from the base of the oesophagus to the region of the posterior ovarian loop. The cuticle is exceedingly finely striated, the striations being most easily seen in the tail region. From the base of the oesophagus, the body gradually becomes attenuated anteriorly to a diameter of about 0.015 to 0.020 mm. The mouth is small and leads directly into the oesophageal lumen. It consists of three indistinct lips with two projections on each, which are presumably papillae; the minute size of these structures renders them exceedingly inconspicuous. The first quarter of the oesophagus is slightly more muscular than the rest; a slight enlargement was observed at the posterior end of the first region. The oesophagus gradually increases in size posteriorly and is from 0.975 to 1.450 mm. long.

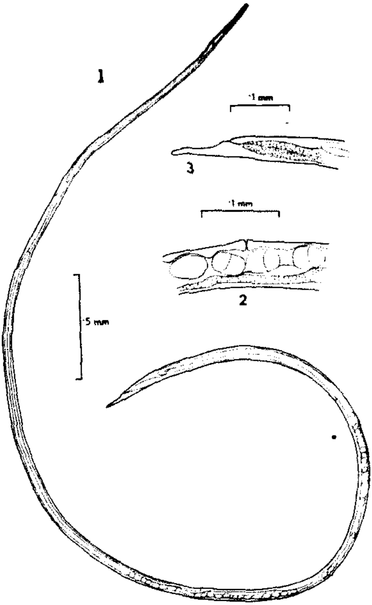
TABLE I

MEASUREMENTS OF 25 SPECIMENS OF *Strongyloides agoutii* SP. NOV.

	Range (n = 25), mm.	Mean, mm.	Standard deviation*	Coefficient of variation, %
Parasitic female				
Total length	3.94 – 6.45	5.170	0.7242	14.0
Width at middle of body	0.03 – 0.059	0.044	0.0063	14.3
Length of oesophagus	0.975 – 1.450	1.210	0.1359	11.2
Length from vulva to tip of tail	1.275 – 2.300	1.750	0.2606	14.8
Length of tail	0.075 – 0.109	0.091	0.0099	10.8
Ova—length, μ	46 – 58	51	3.926	7.6
width, μ	23 – 29	26	1.706	6.5
Free-living male				
Total length	0.870 – 1.050	0.965	0.0617	6.3
Width at middle of body	0.050 – 0.070	0.058	0.0067	11.5
Length of oesophagus	0.120 – 0.160	0.135	0.0096	7.1
Length of tail	0.075 – 0.100	0.086	0.0085	9.8
Length of spicules (10 specimens only)	0.028 – 0.035	0.031	0.0024	7.7
Length of accessory piece (5 specimens only)	0.018 – 0.023	0.021	0.0019	9.0
Free-living female				
Total length	0.975 – 1.350	1.126	0.0930	8.2
Width immediately anterior to vulva	0.045 – 0.075	0.062	0.0075	12.0
Length of oesophagus	0.140 – 0.188	0.156	0.0126	8.0
Length of tail	0.090 – 0.165	0.123	0.0170	13.7
Ova—length, μ	38 – 62	47	4.980	10.5
width, μ	28 – 40	36	3.187	8.8
Filariform larva				
Total length	0.645 – 0.760	0.710	0.0365	5.1
Width at junction of oesophagus and intestine	0.018 – 0.022	0.020	0.0012	6.0
Length of oesophagus	0.276 – 0.354	0.322	0.0252	7.7

* $\sigma = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$.

The longest oesophagus does not necessarily occur in the longest worm, and it may be said that the oesophagus varies from one-quarter to one-fifth of the total length of the body. The vulva (Fig. 2) possesses prominent lips and is situated well posterior to the middle of the body at from 1.275 to 2.300 mm. from the tip of the tail. The gonad is double and consists of an anterior and posterior loop, which may be of the simple hair-pin bend type or may be twisted. No constancy in this character was observed in the material examined. Some specimens showed the simple type in both uteri, while a few showed both loops twisted and others only one loop twisted. The ovaries and uterus occupy most of the body cavity, the bends of the former occurring close to the oesophageal and anal ends of the intestine. Between the posterior ovarian loop and the anus the body commences to taper, becoming consider-



FIGS. 1 – 3. FIG. 1. *Parasitic female.* FIG. 2. *Vulva of parasitic female.* FIG. 3. *Tail of parasitic female.*

ably reduced behind the anus and terminating in a fairly short finger-like tail, broadly rounded at the tip. The tail (Fig. 3) varies in length from 0.075 to 0.109 mm. The eggs are ellipsoidal, thin shelled, and from 46 to 58 μ long by 23 to 29 μ wide. They are not fully embryonated in the uterus, but may contain an embryo at the time of elimination with the faeces. The uteri usually contain several fully developed ova and as many as 25 undeveloped eggs may be seen.

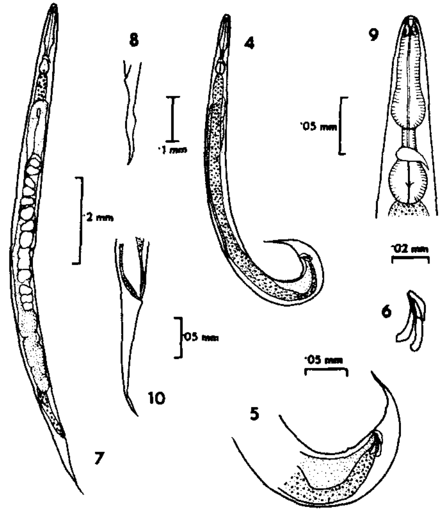
The Free-living Generation

This comprises male and female forms. The latter lay eggs that develop into rhabditiform larvae and subsequently into filariform larvae.

Male (Fig. 4). From 0.870 to 1.050 mm. in length with an average width of 0.058 mm. at the middle of the body, which is of more or less equal diameter except in the tail region. Anterior to the junction of the oesophagus and

intestine the head region tapers uniformly. Anterior to the anus the body shows marked thickening, but posterior to the opening it becomes considerably narrowed, tapering quickly and terminating in a fairly long slender point. The mouth leads into a short pharynx. The oesophagus is from 0.120 to 0.160 mm. long and is made up of two parts connected by a narrow neck. The anterior portion is more or less cylindrical with a slight swelling before narrowing to the neck region. A small area at the anterior end of the oesophagus shows a decided muscular thickening. The neck is fairly long and is followed by a round to flask-shaped, muscular bulb. The nerve ring crosses the neck just anterior to this bulb.

The male gonad is single and extends almost up to the oesophagus, occupying most of the width of the body. The terminal portion of the testis narrows considerably to form a duct leading into the cloacal opening. The tail (Fig. 5) is curved ventrally, and is from 0.090 to 0.165 mm. in length. Caudal papillae are present on the ventral surface though not very conspicuous—one pre-anal and one post-anal approximately equidistant from the anus. The spicules are 0.028 to 0.035 mm. in length and resemble a curved blade



FIGS. 4 – 10. FIG. 4. *Free-living male.* FIG. 5. *Tail of free-living male.* FIG. 6. *Accessory piece and spicules.* FIG. 7. *Free-living female.* FIG. 8. *Abnormal tail of free-living female.* FIG. 9. *Anterior end of free-living female.* FIG. 10. *Tail of free-living female.*

with a knob-like handle (Fig. 6). The accessory piece (Fig. 6) is from 0.018 to 0.023 mm. in length and is of an irregular oval shape.

Female (Fig. 7). From 0.975 to 1.350 mm. in length with an average width of 0.061 mm. immediately anterior to the vulva. Anteriorly the body tapers gradually; posteriorly the narrowing is slightly more abrupt, becoming considerably narrowed in the region of the rectum and tapering finally to a slender pointed tail. The tail was not observed to taper smoothly to a point in all cases; bulbar enlargements (Fig. 8) were observed frequently between the anus and tail tip. The head region and oesophagus of the female (Fig. 9) closely resemble those of the male. The oesophagus is from 0.140 to 0.188 mm. in length. The vulva shows well defined lips and is located slightly posterior to the middle of the body; the gonad is double and consists of anterior and posterior loops of the simple hair-pin bend type. The number of eggs present in the uteri is variable and as many as 23 were observed in well grown females. These ova are more or less sub-spherical in shape with a length of 38 to 62 μ, and a width of 28 to 40 μ. It has been observed that the female has not necessarily finished growing when eggs appear in the uterus. After oviposition is completed the uteri shrink considerably in size. The tail is slender (Fig. 10), pointed, and from 0.090 to 0.165 mm. long.

Rhabditiform larva

The rhabditiform larvae (Fig. 11) show considerable size range, depending on the stage of growth. At the time when the genital primordium is conspicuous, the larvae are approximately 0.4 mm. in length, with a width of 0.02 mm. The genital primordium is located in the region of the middle of the intestine. The tail is slender and tapering.

Filariform larva

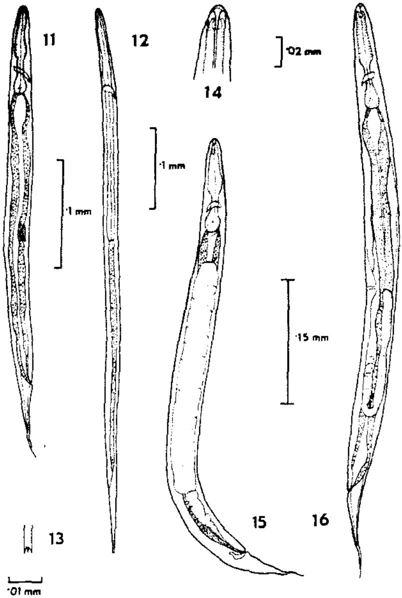
These larvae (Fig. 12) are from 0.645 to 0.760 mm. long and from 0.018 to 0.022 mm. wide in the region of the base of the oesophagus. The oesophagus is from 0.276 to 0.354 mm. long and shows a slight enlargement at the end of its first third; it joins the intestine just anterior to the middle of the body. The genital primordium is located at approximately the posterior end of the first third of the intestine. The tail is straight and tapered, showing a tricuspid termination (Fig. 13).

HOST: *Dasyprocta agouti.*

LOCATION: Small intestine.

TYPE LOCALITY: Trinidad, B.W.I.

The *Strongyloides* from the agouti differs from most of the other species on the basis of size. In this respect it falls between *S. papillosus* and *S. westeri*, though it is never as large as the latter. The majority of specimens are considerably larger than those of *S. papillosus* and the minimum lengths of oesophagus and tail are always greater than the maximum for that species. Moreover, in cross-infection experiments the agouti species will establish



FIGS. 11 – 16. FIG. 11. *Rhabditiform larva (20 hr. old).* FIG. 12. *Filariform larva.* FIG. 13. *Tricuspid tail of filariform larva.* FIG. 14. *Head of immature free-living female.* FIG. 15. *Immature free-living male.* FIG. 16. *Immature free-living female.*

itself readily in the guinea pig but not in the rabbit, whereas this condition is reversed with *S. papillosus.*

Comparative size, shape of tail, as well as the expulsion of embryonated ova in the faeces differentiated this species from *S. stercoralis.* No characteristic spear apparatus was seen in the oesophagus as found in the free-living female of *S. simiae*, and the marked post-vulvar constriction in the free-living females of *S. fülleborni* was not observed. The dimensions of the agouti species distinguish it from the much smaller *S. ratti.*

The twisting of the anterior and posterior ovarian loops does not show any constancy; and the shape of the tail of the parasitic female, although little variation is observed, cannot be considered as a distinctive characteristic.

This species, accordingly, differs from those that have been adequately described and is, therefore, referred to a new species under the name of *Strongyloides agoutii* sp. nov.

Recent Aspects on the Biology of *Strongyloides*

The parasitic generation in the genus *Strongyloides* is represented by females, considered by most authors to be parthenogenetic or hermaphroditic. These females lay eggs that may hatch within the intestine, so that free larvae are found in freshly passed faeces. In some species, however, the eggs themselves appear in the faeces. The larvae that are discharged in the faeces or hatched from the ova are known as rhabditiform larvae. These may metamorphose directly to the filariform or infective larvae or they may develop into a free-living bisexual generation of rhabditiform males and females. The free-living sexual generation produce fertile ova which give rise to rhabditiform larvae and later to infective filariform larvae. The former metamorphosis is known as the direct or homogonic type of development, the latter as the indirect or heterogonic method.

There are thus two possible cycles in the life history of members of this genus which permit alternative modes of origin of the infective filariform larvae. Since the occurrence of these two modes of development was confirmed, many hypotheses and theories concerning the factors influencing them have been offered.

Earlier workers considered that physical, chemical, and mechanical factors in association with environmental conditions, might influence the mode of development, but investigation did not confirm these views.

Recent workers, by the use of cultures in which both types had appeared, have been unable to produce changes in either type by subjection to controlled environmental conditions. The hypothesis that the direct developing type was confined to the temperate zone and the indirect to the tropics was accepted for some time (24, 25). Further suggestions were made that the differences in mode of development might be attributed to nutritional conditions to which the larvae are subjected prior to leaving the intestine of the host (Darling, 1911).

More critical methods of procedure and experimentation have proved the former theories to be untenable. Pure strains of one type were seldom observed and the general instability of types was accepted. The infection of certain abnormal hosts was found to lead to changes in the type of life cycle. Cytological studies of the gonads of the parasitic female led to the belief that the female is really hermaphroditic rather than parthenogenetic (28–31). This observation gave rise to a theory that the direction of development is determined by the chromosome constitution of the eggs after fertilization, and that the sex of the rhabditiform generation is determined according to whether the eggs are fertilized by sperm bearing, or not bearing, a heterochromosome (32).

The classical life cycle of the genus *Strongyloides* was generally accepted until comparatively recent years. In addition to the direct and indirect phases of development of *Strongyloides*, a distinct hyper-infective type was stated to occur, resulting from the rhabditiform larvae metamorphosing to

infective larvae prior to feeding (6, 7). This type is, therefore, the form responsible for the so-called "auto-infection" or "hyper-infection" of individuals that have become parasitized previously. At the same time, the administration of gentian violet as a strongyloidicide was believed to change the indirect and hyperinfective strains of *Strongyloides* to the direct type, a factor of no little importance to the fundamental biological ideas of the life cycle of the genus (8).

The discovery of male parasitic stages gave rise to further hypotheses concerning the mode of development of the free-living generation (9, 23). Evidence has been presented in support of the view that spermatozoa observed in the reproductive tubule of the female are the result of a process of insemination by a male. It is suggested that this process occurs during the latter part of the adolescent stage of the female, and that the insemination may take place in the lungs (where females and males have been observed together) prior to the migration of the female into the mucosa of the intestinal wall. This insemination is considered to suffice for several months after the females invade these tissues. The belief that fertilized ova give rise to an indirect type, while unfertilized ova may produce a direct type of development, was put forward. This idea is consistent with the tendency of strains that have dwelt within a host for any length of time to change from indirect to direct type as the supply of spermatozoa becomes depleted.

Experimental evidence has been presented to show the continued propagation of the free-living generation on artificial media (1, 2). It has been stated that when a known species of the genus, which most nearly demonstrates a typical indirect mode of development, is cultured under optimum conditions, it will continue to develop indirectly. However, when subjected to unfavourable environmental conditions (e.g., reduction in quantity or quality of nutrient or reduction in viscosity) a modification of the strain toward a direct type may be observed. Free-living adults of the first, second, and third generations were grown on artificial media; and it is believed that continued propagation of the free-living sexual generation occurs normally in nature under optimum conditions. This evidence would tend to suggest that "directness" and "indirectness" are conditioned by environment and not by the "genetical make up" of the ovum.

The biological studies on the genus during the past few years have tended to complicate rather than simplify the picture. As a result of critical investigations involving the transfer of a pure line strain of *S. ratti* through 14 parasitic generations by means of single larva transfers, it has been demonstrated that a male parasite is unnecessary in the bionomics of *S. ratti* either to maintain parasitic fertility or to explain the two modes of larval development (16–18). The theory of hyper-infection as a possible source of either hypothetical parasitic males or additional parasitic females is not supported, and the parasitic phase of *S. ratti* is considered to be represented by a female organism either syngonic or parthenogenetic.

It has recently been shown that the frequency with which the daily yield of offspring (resulting from singly established parasites of *S. ratti* in the rat) included adults of heterogonic development was subject to fluctuations over long seasonal cycles. Data suggest that the rat responds physiologically to changing meteorological conditions and that the altered environment thus produced for *S. ratti* leads to changes in the relative frequency with which progeny of heterogonic development are produced. All evidence from these recent studies indicates that the mode of larval development shown by the progeny of *S. ratti* is determined prior to oviposition (19).

The Free-living or Sexual Generation of *Strongyloides agoutii*

It was considered advisable to continue observations of development of the sexual generation over as long a period as possible, in order to record any appearance of latent factors that might not reveal themselves in a short time. The observations extended from April 1936 until March 1939, some 139 cultures being made. The collection of at least three faecal samples per month seemed desirable, and as a general rule these were collected on different days; on occasions when cultures failed to furnish a sufficient nematode population, further samples were collected during that month. In this way, it was considered that any seasonal variation in development would be observed.

All cultures during 1936 were kept at room temperature, which ranged from 15 to 30° C., with a usual temperature of 23° C. During the first six months of 1937 the temperature was held regularly at 22° C., though on 15 occasions in May and June it ranged between 25 and 30° C. During the latter part of 1937 and throughout 1938 and 1939 the temperature variation was similar to 1936.

The routine procedure adopted in examination was modified to suit each set of cultures. However, since with this species ova are deposited in the faeces, the first examination of all cultures was made on the morning following the day of collection, usually 20 hr. after passage; subsequent examinations were daily or oftener, as necessary. If only filariform larvae were present on two or more consecutive examinations, the daily observations were discontinued and cultures were examined on alternate or on every third day. As the Petri plate lids were not lined with filter paper, it was usually necessary to moisten cultures at least once a week. Sufficient aeration was provided by removal of the lids when examination was carried out under the binocular microscope. In most instances, developmental forms could be differentiated under 16 to 32 magnifications, but if doubtful they were examined under the compound microscope. Cultures were discarded when two or more consecutive examinations showed no activity.

It will be observed that the total number of cultures recorded on the graphs does not correspond to the total examined in the investigation. A few records have been deleted when the culture has been rendered inactive soon after

origin, because of abnormal conditions; in some, larval development was not observed; in a few, all the larvae were used for experimental infection purposes. During the three-year period, time lost by sickness or holidays was responsible for the discard of some cultures. The analysis of these cultures shows the indirect or heterogonic type of development was consistent over the three years, showing no variation from month to month.

As a general rule, rhabditiform larvae were not observed on first examination of the faeces. In the majority larvae were not observed in any number for 48 hr., after which immature males and females were observed. In all instances females predominated in numbers, males being present in most but always in a great minority. The length of life of the male was considerably shorter than that of the female, the former dying after the second or third day of activity.

From Fig. 17 it will be seen that most free-living sexual forms appeared between the second and fifth days of culture and disappeared over a period of from 3 to 11 days. Free-living sexual stages disappeared from most cultures by the seventh or eighth day, although in four cases they lived as long as 11 days.

In Fig. 18 the first appearance of filariform larvae is indicated. In many cultures filariform larvae appeared on the fourth day, though in the greatest number they appeared on the sixth day; by the eighth day, larvae were present in all cultures.

The criterion of life of cultures is the activity of filariform larvae. From Fig. 19 it is seen that in the greatest number of cultures larvae became inactive between the 21st and 25th days. They remained active in some for 35 to 40 days, and in one only for 41 days. From Fig. 18 it is seen that filariform larvae appeared in the majority by the sixth day. Accordingly, the maximum duration of life of the filariform larvae is 35 days.

The Continued Propagation of the Free-living Generation of *Strongyloides agoutii*

The first intensive study of this nature with a member of the genus *Strongyloides* was made by Beach (1, 2). With both human and primate species of *Strongyloides*, he recorded continued propagation of the free-living phase of *S. simiae* on artificial culture media for three generations. His extensive investigations show that *S. simiae*, which follows the totally indirect type of development, changed to a mixed type when cultured on various artificial media. Parthenogenesis in the free-living female of this species was not found to occur. A single parasitic female, placed in half-strength Locke's solution, laid eggs that developed into males and direct filariform larvae in three cultures, while males, females, and filariform larvae developed in another.

Observations on the filariform larvae of *S. fülleborni* in different media as well as in tissue culture were recorded by Chung (5). In most instances there was no development, though he found that larvae survived for many

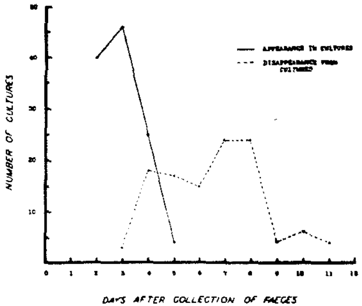


FIG. 17. *Appearance and disappearance of free-living sexual stages of S. agoutii in faecal cultures from agouti.*

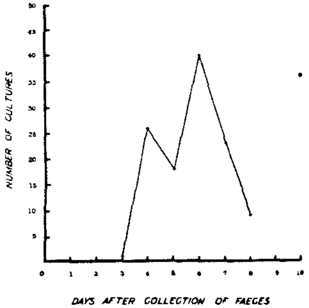


FIG. 18. *Appearance of filariform larvae of S. agoutii after passage of faeces by agouti.*

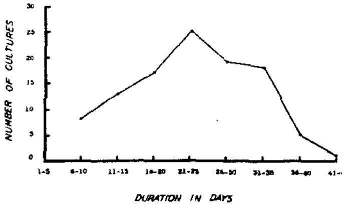


FIG. 19. *Longevity of filariform larvae of S. agoutii in faecal cultures from agouti.*

days in a medium having a pH between 5.4 and 7.0; a pH above 7.8 was unfavourable. Kouri, Basneuvo, and Arenas (22) report that with *S. stercoralis*, after numerous free-living generations, a change to an entirely free-living individual takes place, the female becoming parthenogenetic and there being no males. The fecundity of these females gradually decreases until the cultures become sterile. The findings of Beach (2) indicate that directness and indirectness in development of the free-living phase of the life cycle is contingent on environmental factors only. He considers that optimum conditions tend to produce the indirect type of development, whereas unfavourable conditions produce the direct.

In order to ascertain if continuous propagation of *S. agoutii* could be induced in artificial culture, a series of experiments was made.

To permit detailed observation of isolated forms in culture, a glass culture slide with a well 16 mm. in diameter by 3 mm. deep was employed. Small Petri dishes were also found satisfactory when it was desired to culture several individuals together. The medium used was similar to that used by Beach, with substitution of extract of agouti faeces in place of monkey faeces, and 1 gm. instead of 2 gm. of agar. It consisted of the following: 1 gm. of nutrient agar, 25 cc. of aqueous extract of agouti faeces, 75 cc. of distilled water. It was sterilized at 15 lb. for 20 min. to ensure the killing of any ova or larvae that might have been present. A thin film was placed on the bottom of the Petri dishes or of the well in the slides. This thin layer permitted the use of the compound microscope for examination purposes.

Larvae were obtained by Baermanning cultures of agouti faeces. Mature males and females were then selected. No attempt was made to control bacterial development in the cultures, but colonies were observed in only a few instances. It was necessary to moisten cultures quite frequently even though the slides were kept over water in large glass culture dishes, the lids of which were lined with filter paper to avoid excessive evaporation. Cultures were examined daily. Some 30 isolation cultures were made and examined in sets of six. The first group was cultured at room temperature of approximately 23° C. Slides No. 1, 2, and 5 were allotted two mature females each; No. 3, one immature female; No. 4, one female and one male; and two males to No. 6. With the exception of No. 1 and 4, the free-living stages were all dead by the fourth day; No. 1 and 4 showed active filariform larvae until the 18th and 10th days respectively.

Group 2 was cultured at 30° C. Slides were prepared with the following combinations: No. 7 with one male; No. 8 and 9 with a female each; No. 10 with a female and a male; and No. 11 and 12 with two females each. All individuals in this group were dead by the fourth day and no progeny was recorded.

Group 3 was cultured at room temperature. Slides No. 13–15 were made up with one female and one male each; No. 16 and 17 with two females each; No. 18 with two males. The males on No. 18 were dead by the third day.

Filariform larvae were observed in the other cultures and the duration of activity noted was 5, 6, 7, and 18 days.

Group 4 was cultured at 25° C. Slides No. 19–22 were allotted one male and one female each. No. 19 showed active filariform larvae up until the 11th day; No. 21 showed larvae until the fifth day; No. 20 and 22 produced no progeny. Progeny of No. 23, which comprised two females, were active until the 18th day; and the two males of No. 24 lived but three days.

Small Petri plates were used for cultures No. 25 to 30. The first three contained five females and one male each, the others, five females and five males each. The cultures were kept at room temperature (23° C.) and filariform larvae developed, but all activity ceased on the seventh and eighth days.

Discussion

A morphological and biological study of a hitherto unrecorded member of the genus *Strongyloides* has justified the creation of a new species, *S. agoutii*.

Variation in rate of growth and maturity of free-living forms was observed to occur, the regulating factor probably being food supply or environmental conditions of the media. The wide range of results, presented graphically, was unavoidable both on account of the actual error in observation and the excessive rate of metamorphosis of forms from one stage to another.

The frequency of the first appearance of filariform larvae on the fourth day might possibly be interpreted as the appearance of direct development in cultures. This occurrence could conceivably have been overlooked or not differentiated by the technical methods employed in routine observations. However, since the direct type of development was never observed, it seems improbable that it occurred.

From the data obtained, there is no evidence of continued propagation of the free-living generation of *S. agoutii*.

Acknowledgment

This investigation was undertaken at the Institute of Parasitology, Macdonald College, under the supervision of the Director, Prof. T. W. M. Cameron, who brought the agouti from Trinidad. The author wishes to express his appreciation and thanks to Professor Cameron for his continued interest, guidance, and generous assistance at all times throughout this study.

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CANADIAN WILTSHIRE BACON

V. QUANTITATIVE BACTERIOLOGICAL STUDIES ON CURING PICKLES[1]

By N. E. Gibbons[2]

Abstract

Five combinations of diluent, salt concentration of medium, and incubation temperature have been used to study the bacterial content of curing pickles used for making Wiltshire bacon. On representative pickles from 16 plants the highest mean count was on 10% salt agar (brine dilution) incubated at 20° C. The lowest count was on nutrient agar incubated at 37° C. Counts on media containing no salt, 4% salt, and 10% salt (water dilution) with incubation at 20° C. gave intermediate values. Counts on spent pickles were higher than on cover pickles. Pump pickles showed a surprisingly high number of organisms.

The analytical error attributable to diluting, plating, and counting was relatively small compared with the other sources of variance. Under certain conditions the error of sub-sampling a small jar of pickle exceeds the error between replicate plates. Sampling and sub-sampling errors were therefore the primary factors limiting the precision of the determinations.

Statistical analysis of the results showed that the differences in numbers observed by the different methods were highly significant for all pickles from 16 plants, and that the bacterial content of the pickles from the different plants differed significantly over all media. The number of bacteria observed by the different methods was usually correlated, i.e., pickles from a plant showing a high count by one method usually showed a high count by the other methods and vice versa. Nevertheless, it was possible to demonstrate a significant differential response of the bacteria in cover and spent pickles to the different growth conditions used. This suggests qualitative differences in the flora of the pickles. Of the several growth conditions tested, nutrient agar at 20° C. and 10% salt agar with brine dilutions appear to be the most suitable for demonstrating differential responses attributable to qualitative differences between the flora of different pickles.

Introduction

An outline of the investigation on Wiltshire-cured bacon has been given in a previous paper (2). The present paper deals with the quantitative bacteriological findings on representative curing pickles obtained from the various plants. Several combinations of growth conditions were used in order to obtain, in addition to the strict quantitative variations in bacterial populations, an estimate of the validity of this procedure for detecting qualitative differences in the bacterial flora of different plants.

Samples of the three pickles used for curing were obtained from each plant. The pump pickle, injected into the sides prior to cure, is usually a freshly prepared brine which might reasonably be expected to contain few bacteria. The tank pickle, used to cover the sides during cure, was analysed at the beginning and end of cure, these samples being designated "cover" and "spent" pickles respectively. Details of the design of the experiment and methods of

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[2] *Bacteriologist, Food Storage and Transport Investigations.*

sampling and of shipping the pickle have been described previously (2). Most pickles were sampled within an hour or two of receipt, although occasionally it was necessary to hold them as long as 12 hr. Pump pickles from 19 plants, and cover and spent pickles from 16 plants, furnished the data on which this paper is based.

Statistical methods were used to reduce and assist in the interpretation of the data (6). Since the bacterial numbers showed great variation, both between plants and the various cultural methods employed, the logarithms of the numbers were used both for convenience and valid interpretation on a statistical basis (1).

Cultural Methods

Lochhead (5) has shown that the count obtained on nutrient agar with incubation at 37° C., as commonly used in most packing plants for control purposes, is lower than that on nutrient agar or salt agar incubated at 20° C. Nutrient agar containing 10% salt, following dilution in brine of the same concentration, gave the highest counts, these being about 80 times greater than those obtained on nutrient agar incubated at 37° C. Water dilutions were found to render up to 90% of the organisms in pickle incapable of growth.

TABLE I

METHODS USED TO ESTIMATE THE NUMBER OF BACTERIA IN CURING PICKLES

Method	A	B	C	D	E
Temp. of incubation, °C.	37	20	20	20	20
Diluent	Dist. water	Dist. water	Dist. water	4% NaCl*	10% NaCl*
Medium	Beef-extract agar	Beef-extract agar	Beef-extract agar + 10% NaCl	Beef-extract agar + 4% NaCl	Beef-extract agar + 10% NaCl
Period of incubation, days	3	7	10	7	10

* *% = Gm. added to 100 ml. of liquid.*

These facts formed the basis for adopting the methods outlined in Table I. Method A was selected since it is commonly used for control purposes in packing plants. As a working hypothesis it was assumed that Method B would yield some estimate of the organisms introduced by the fresh sides; Method C, the salt-tolerating organisms capable of withstanding considerable change in osmotic pressure; Method D, the organisms capable of rapid growth on cured bacon; and Method E, the halophilic flora of the pickle. It is recognized that these methods may overlap considerably and that they do not yield an accurate estimate of the various types present. They were, however, considered suitable for a preliminary investigation of the subject.

Sampling

Since it was impossible to visit each of the plants, the samples were taken by a plant operator, who was provided with detailed instructions. By obtaining two samples of the pickle (taken at different times) from each plant,

it was possible to obtain an estimate of the combined error due to sampling and to any differential change that occurred in the samples during shipment. A discussion of this phase of sampling has been given (2).

Apart from any systematic change that may occur in the bacterial numbers during shipment at different temperatures over varying periods of time, it is of interest to point out that a sub-sampling error arising from the removal of duplicate samples from a small jar of pickle was greater in some instances than the analytical error of plating and counting. The results of typical experiments appear in Table II. It can be seen that the sub-sampling error is significantly greater than the analytical error in two experiments at 22° C. and approaches significance in the third. Of the six experiments at 1.1° C., three of which were shaken more frequently during storage than the others, in only two was the sub-sampling error significantly greater. In both instances the variance necessary for significance was contributed by one set of duplicates. Since this error is apparently greater when pickle is stored at the higher temperature, it is possible that it is the result of protein precipitation which interferes with the uniform distribution of the organisms through the sample.

TABLE II

SAMPLING AND ANALYTICAL ERRORS OF COUNTS ON PICKLES STORED UNDER DIFFERENT CONDITIONS

Pickle	Method	Storage temperature, °C.	*Sampling and analytical error*		*Analytical error*		*F*
			D.f.	Mean sq.	D.f.	Mean sq.	
1	B	22	7	.0817	14	.0062	13.18**
		1.1	6	.0178	12	.0083	2.14
		1.1 (shaken)	6	.0148	12	.0099	1.49
2	B	22	7	.0249	13	.0094	2.65
		1.1	6	.0021	12	.0041	0.51
		1.1 (shaken)	6	.0047	12	.0012	3.77*
	E	22	7	.0378	28	.0044	8.59**
		1.1	6	.0086	24	.0027	3.18*
		1.1 (shaken)	6	.0091	24	.0177	0.51

* *Indicates 5%, ** 1% level of significance.*

Quantitative Results

Typical arithmetic counts of cover pickles by the various methods are shown in Table III. The logarithms of the mean, maximum, and minimum number of bacteria per ml. over all plants are given in Table IV. In general the counts were maximal on 10% salt agar (Method E) and decreased with decreasing salt concentration (Methods D and B) and with increasing temperature (Method A). Dilution with water and incubation on 10% salt medium (Method C) gave results comparable with Method B. The mean

TABLE III

REPRESENTATIVE BACTERIAL COUNTS ON COVER PICKLES UNDER DIFFERENT CULTURAL CONDITIONS. NUMBER OF ORGANISMS PER ML.

Sample	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	Dist. water 10% salt agar 20° C. (C)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)
1	700	5,000	7,400	60,000	900,000
2	75,000	96,000	57,000	300,000	470,000
3	12,000	36,000	19,000	61,000	120,000
4	46,000	150,000	170,000	900,000	4,700,000
5	70	600	500	770	600
6	630	12,000	11,000	30,000	34,000
7	39,000	130,000	63,000	500,000	490,000

TABLE IV

BACTERIAL COUNTS OF CURING PICKLES UNDER DIFFERENT CONDITIONS OF GROWTH

	Logarithm of number of organisms per ml.				
	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)	Dist. water 10% salt agar 20° C. (C)
Pump pickle					
Mean	3.01	3.69	4.25	4.40	3.78
Maximum	4.65	4.89	5.40	5.98	4.89
Minimum	1.68	2.40	2.43	2.15	2.13
Stand. dev.	.71	.65	.88	1.10	.78
Coeff. of variation	23.6	17.6	20.7	25.0	20.6
Cover pickle					
Mean	3.63	4.36	4.96	5.33	4.29
Maximum	5.00	5.36	6.24	7.12	5.48
Minimum	1.14	2.11	2.23	2.46	2.21
Stand. dev.	1.09	.87	1.07	1.23	.87
Coeff. of variation	30.0	20.0	21.6	23.1	20.3
Spent pickle					
Mean	4.44	4.99	5.68	5.95	4.88
Maximum	5.22	5.78	6.89	7.15	5.87
Minimum	3.26	3.85	4.35	4.47	3.68
Stand. dev.	.51	.44	.56	.66	.51
Coeff. of variation	11.5	8.8	9.9	11.1	10.4

number of bacteria in cover and spent pickles from all plants shows that Methods A, B, and D measure about 2.5, 10, and 50%, respectively, of the number observed by Method E. This agrees quite well with Lochhead's figures of 1.3, 5.7, and 52.3% obtained from a number of observations on the curing pickle of one plant (5).

On all media the mean count increases in the order, pump, cover, and spent pickle. It is interesting to note that the freshly prepared pump pickle contains, on the average, almost as many organisms as the cover pickle. In fact,

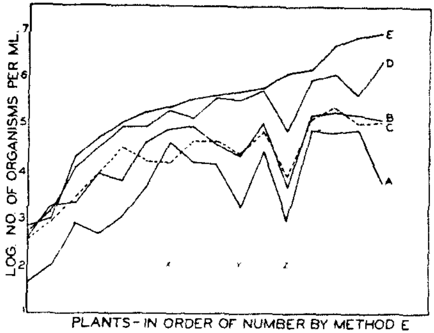

FIG. 1. *Bacterial content of cover pickle from 16 plants as indicated by different cultural methods: A—nutrient agar incubated at 37° C.; B—nutrient agar incubated at 20° C.; C—10% salt agar from water dilutions; D—4% salt agar; and E—10% salt agar from brine dilutions. All incubated at 20° C.*

the minimum values for pump pickle are usually higher than those for cover pickle.

The maximum and minimum values, the standard deviation and the coefficient of variation (Table IV) give some indication of the variation that occurs between the various counts and pickles. Since the standard deviation usually increases with the mean number present by each method, the coefficient of variation is relatively constant for each type of pickle. The standard deviation and coefficient of variation indicate that counts on nutrient agar at 20° C. are the least variable, both absolutely and relatively, while the 10% salt agar count is relatively the most variable for pump pickle and the 37° C. count relatively most variable for cover and spent pickles. It is evident that the spent pickle is the least variable of the three types. It would seem that the number of bacteria present in cover pickle reflects the many variations in pickle formula and plant practice, but during cure becomes more uniform.

Sources of Variability

The variability indicated by the standard deviation in Table IV can be divided into that originating from three main sources: (i) combined error of dilution, plating, and counting (analytical error); (ii) combined error of sampling the tank, differential changes during shipment, and sub-sampling the jar of pickle (sampling error); and (iii) the difference in count of pickles from different plants. Since the number of organisms in the pickle may vary from time to time in the same plant, the sampling error is probably overestimated.

The analysis of variance of the counts on the three types of pickle is shown in Table V. In all cases the sampling error was significantly greater than

TABLE V

ANALYSIS OF VARIANCE OF BACTERIAL COUNTS ON PICKLES

Variance	Dist. water nut. agar 37° C. (A)		Dist. water nut. agar 20° C. (B)		4% brine 4% salt agar 20° C. (D)		10% brine 10% salt agar 20° C. (E)		Dist. water 10% salt agar 20° C. (C)	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump pickle										
Analytical error	71	.0042	72	.0014	74	.0020	72	.0023	74	.0020
Sampling error	19	1.1564**	19	.4593**	19	.3645**	19	.5663**	19	.5337**
Between plants	18	1.8093	18	2.0360**	18	4.3358**	18	6.7464**	18	3.1623**
Cover pickle										
Analytical error	64	.0142	61	.0022	62	.0012	61	.0026	61	.0034
Sampling error	16	1.1579**	16	.6007**	16	.2541**	16	.8702**	16	.8003**
Between plants	15	6.3017**	15	4.0321**	15	6.0120**	15	8.2778**	15	3.7652**
Spent pickle										
Analytical error	59	.0019	62	.0028	61	.0049	61	.0056	61	.0016
Sampling error	16	.3377**	16	.2597**	16	.2468**	16	.2741**	16	.2958**
Between plants	15	1.2070**	15	.9020**	15	1.6128**	15	2.3336**	15	1.2660**

*** Indicates 1% level of significance.*

the analytical error. Most of the variance attributable to sampling was contributed by a few plants showing considerable differences between the two samples. In spite of the large sampling error the difference between the bacterial numbers present in the pickles from different plants was highly significant in all but one instance. It can therefore be said with assurance that the bacterial numbers present in the pickles vary much more between plants than within plants, since the latter source of variance is included in the sampling error.

Relations of Counts by Various Methods

The numerical differences observed by the various methods may result from (i) essentially similar floras in all pickles with a fixed proportion of the maximum observed population developing under the different conditions, or (ii) different floras with a different fraction of the total population favoured by each method. Obviously a high degree of correlation between the quantitative counts by the several methods would indicate the first condition, while no correlation would suggest the second. Preliminary examination of the data indicated that there was some evidence favouring both of the above behaviours. For instance, a pickle having a high count by one method usually had high counts by all methods, suggesting a correlation between them. On the other hand, although the number of bacteria observed on nutrient agar incubated at 37° C. was on the average 2.5% of that observed on 10% salt agar incubated at 20° C., for the individual pickles this proportion varied from 0.08 to 16%. Although a general association exists between the bacterial numbers observed by the different methods for each cover pickle,

TABLE VI

CORRELATION BETWEEN NUMBER OF BACTERIA GROWING ON VARIOUS MEDIA

Quantities correlated			Correlation coefficient (r)		
			Pump pickle[1]	Cover pickle[2]	Spent pickle[1]
Number on nut. agar 20° C.	*and*	Number on nut. agar 37° C.	.92	.94	.85
		4% salt agar	.89	.91	.90
		10% salt agar	.86	.86	.77
		10% salt agar (water)	.94‡	.93	.92
Number on 4% salt agar	*and*	Number on nut. agar 37° C.	.74†	.90	.81
		10% salt agar	.97‡	.91	.81
		10% salt agar (water)	.94‡	.96	.91
Number on 10% salt agar	*and*	Number on nut. agar 37° C.	.72†	.81	.70
		10% salt agar (water)	.94‡	.92	.83
Number on nut. agar 37° C.	*and*	Number on 10% salt agar (water)	.83	.87	.80

[1] *17 degrees of freedom, r of .58 required for 1% level of significance.*
[2] *14 degrees of freedom, r of .62 required for 1% level of significance.*
‡ *Significantly different from †.*

as shown in Fig. 1, there are some wide fluctuations as indicated when the numbers obtained from the plants marked y and z are compared with those from plant x.

In order to place these observations on a more quantitative basis, correlation coefficients were computed between the numbers of bacteria observed by the different methods. The values obtained appear in Table VI. All of these coefficients are statistically significant, thereby demonstrating a definite association between the numbers of bacteria indicated by the different methods. In other words, a pickle having relatively large bacterial numbers by one method will generally have relatively large numbers by all methods. On the other hand, the magnitude of the correlation coefficients is such that the residual variance unaccounted for by the coefficient varies from 5 to 50% of the total variance. This shows that there is a considerable element of independent fluctuation between the numbers observed by the different methods. These independent fluctuations responsible for the residual variance may be attributable wholly, or in part, to experimental error, or, as suggested earlier, to the possibility that the various cultural methods are capable of distinguishing to some extent a real difference between the floras present in different pickles. In order to determine the significance of these observations it was necessary to determine the differential response of the bacterial population to different cultural methods in a manner which permitted comparison with the known experimental errors. This suggested a variance and regression analysis of the data.

The differential response of the flora in the different pickles to the different cultural conditions is included in the interaction mean square, plants × methods (Table VII). This interaction should be tested for significance by comparing it with the variance between aliquots. Since duplicate determinations were not made on each medium this comparison was impossible. However, it has been shown (Table II) that for pickles kept at low temperatures the error between aliquots (sampling and analytical error) was seldom significantly greater than the analytical error alone, and in consequence the largest analytical error observed was used for comparison. On this basis the interaction mean square was found to be overwhelmingly significant.

TABLE VII

VARIANCE AND REGRESSION ANALYSES OF COUNTS BY ALL METHODS

Nut. agar 37° C.; nut. agar, 4% and 10% salt agar at 20° C.; 10% salt agar from distilled water at 20° C.

Variance	Pump		Cover		Spent	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Between plants	18	15.9807**	15	11.3190**	15	6.3109**
Between media	4	33.5442**	4	94.6674**	4	36.2393**
Differential plants × media	72	.6703**	60	.7307**	60	.3137**
Greatest analytical error	71	.0042	64	.0142	61	.0056
Greatest sampling error	19	1.1564	16	1.1579	16	.3377
Regression						
Differences in regressions	18	12.2352**	15	8.4022**	15	3.5119**
Residuals	57	1.2185	48	2.8537	48	1.2554

** *Indicates 1% level of significance.*

The fact that this plants × media interaction derived from an analysis of variance was statistically significant does not provide definite evidence that the organisms present in the different pickles respond differentially to the different growth conditions. The magnitude of the interaction may be affected by the number of organisms present in the pickle as well as by differential effects attributable to differences in flora. Since the number of organisms in the different pickles has been shown to differ significantly, it is necessary to determine the true differential effect from a regression analysis (7). It was then found (Table VII) that the differences between regressions accounted for the major portion of the interaction variance in pump pickle. There is, therefore, little evidence to indicate that different types of floras are present in this pickle. For cover and spent pickles the variance due to differences in regressions, although significant, accounts for only about half the interaction variance. The remainder is therefore attributed to differential response of the organisms to different growth conditions. This finding suggests some difference in the types of organisms present in the different pickles.

The various cultural methods included the effect of salt concentration, temperature of incubation and dilution with water and 10% brine. A further study was made to determine which of these factors contributed the most variance to the observed interaction (Table VII). The results, appearing in Table VIII, show that an increase in salt concentration from 0 to 10% in the medium contributes significantly more to the interaction than increasing the incubation temperature from 20° to 37° C. with a salt-free medium. The effect of dilution method (water or 10% brine) has a smaller differential effect than the salt concentration in the medium but a somewhat greater effect than the incubation temperature.

TABLE VIII

INTERACTIONS OF PAIRED METHODS SHOWING EFFECT OF TEMPERATURE OF INCUBATION, AND SALT CONTENT OF MEDIUM AND DILUENT

		Effect of					
		Temperature of incubation		Salt conc. of medium		Salt conc. of diluent	
		D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump	Interaction	18	.1722†	18	1.2458‡	18	.6294‡
	Greatest anal. error	71	.0042	72	.0023	72	.0023
Cover	Interaction	15	.4259†	15	1.2377‡	15	.9579
	Greatest anal. error	64	.0142	61	.0026	61	.0034
Spent	Interaction	15	.1650†	15	.5304‡	15	.9553‡
	Greatest anal. error	62	.0028	61	.0056	61	.0056

All interactions significant to 1% level.
‡ *Significantly different from* †.

These results show that the bacteriological difference between corresponding pickles from different plants is mainly quantitative. Cover and spent pickles, however, also show some differential response when cultured by different methods, suggesting qualitative differences in the flora. Media containing no salt and 10% salt incubated at 20° C. appear to be the most effective for demonstrating this differential response.

These findings are in agreement with practical conclusions. Since pump pickle is made in all plants from similar ingredients, quantitative rather than qualitative differences might be expected between different plants. However, because of the many methods of reclaiming spent pickle and the varying sources of contamination for cover pickle some differences in the types of organisms found in these pickles in different plants are possible.

Correlation of Bacterial Count with Chemical Composition of Pickles

Since bacterial activity is considered responsible for some changes in pickle composition during cure, correlation coefficients were computed between the differences in the logarithms of the number of bacteria present in the cover and spent pickles, or the relative growth rate during cure, and several constituents of the pickle (3). The correlations between the growth rate observed

in 4 and 10% salt media incubated at 20° C. and the mean value of certain components of the pickles appear in Table IX. None of these correlations was statistically significant for the 15 degrees of freedom available. Nevertheless the coefficient between pH and the relative growth rate was high enough to suggest some relation, the growth rate increasing as the pH increases within the range of observed values (4). Computation of this correlation on the basis of the results from individual pickles did not yield significant values, although twice as many degrees of freedom were available. Correlations between the arithmetic increase in bacterial numbers during cure and the nitrate and nitrite contents and the pH of the pickles were also insignificant.

TABLE IX

CORRELATION BETWEEN BACTERIAL GROWTH RATE AND MEAN VALUE OF CHEMICAL CONSTITUENTS OF COVER AND SPENT PICKLES

Quantities correlated			Correlation coefficient (r)
Log difference of counts on spent and cover pickles (4% salt agar)	*and*	Salt	−.01
		Nitrate	−.12
		Nitrite	.02
		pH	.45
		Protein	−.33
Log difference of counts on spent and cover pickles (10% salt agar)	*and*	Salt	.19
		Nitrate	.22
		Nitrite	.11
		pH	.36
		Protein	−.01

15 degrees of freedom, r of 0.48 required for 5% level of significance.

Actually a correlation with salt content could hardly be expected. The salt content of pickle varies from about 22 to 28%, and for halophilic organisms variations in salt content at concentrations about 20% have little effect. On the other hand, organisms affected by salt concentration are usually inhibited by concentrations much below this. A correlation between the increase in the number of bacteria, or of nitrate reducing organisms, and the total nitrate reduced might be expected, but under plant conditions it is very difficult to differentiate between the nitrate reduced and that absorbed by the sides since the combined errors applicable to the several determinations are large compared with the quantity of nitrate reduced. Likewise a measure of the total nitrite formed is difficult since the amount that reacts with the muscle proteins is unknown. Until these total quantities can be determined correlations of chemical constituents and bacterial numbers of pickle seem unlikely.

Acknowledgments

In addition to the managements of the various plants and those acknowledged in the first paper of this series, the author is indebted to Drs. J. W. Hopkins and W. H. Cook for advice in making and interpreting the statistical analyses, and to Mr. A. Morrison for assistance in making the counts.

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CANADIAN WILTSHIRE BACON

VI. QUANTITATIVE BACTERIOLOGICAL STUDIES ON PRODUCT[1]

By N. E. Gibbons[2]

Abstract

Surface counts of bacteria on the ribs of bacon showed that nutrient agar containing 4% salt incubated at 20° C. gave the maximum number. In sampling the surface of a side of bacon, it was found that the removal of the surface layer of tissue yielded more accurate values than methods based on the removal of organisms by swabs or filter paper impressions.

On the average, the bacterial load on the anterior ribs was greater than on the posterior ribs. Although there were significant differences in the number of bacteria on sides from the same plant, the greatest variation was between sides from different plants.

A visible growth of bacteria or "slime" becomes evident on the average when the logarithm of the number of organisms per sq. cm. exceeds 7.2. Nevertheless, certain sides may appear slimy at log 6.7 per sq. cm., while others will not show this condition at log 8.0 per sq. cm. This variation in the number of organisms present at the visible slime level may result from differences in the flora, different types of growth of the same organism, or variability in the method of detection.

The number of bacteria on the side was found to be correlated with the age of the sides from cure or from packing. The growth rate is slow during the first 8 to 10 days from packing, after which it increases. Sides having an initial load of 100,000 organisms per sq. cm. at packing may be expected to remain free from slime for 20 to 25 days, if stored at 1.1° C. No correlation was obtained between the number of bacteria in the curing pickle and the number on the product.

Introduction

Wiltshire sides are matured for a week to ten days after removal from the curing tank. Whether bacteria play a part in this process is not known, but there is a development of organisms on the surface of the meat which eventually may lead to undesirable features, such as slime or taint.

This study was undertaken to determine the variation in the bacterial load on sides of factory-cured Canadian Wiltshire bacon at the time of export, and the effect of transport or storage on the increase in bacterial numbers and the appearance of slime. For this purpose two sides from each of 22 plants were examined. The treatment of these sides, both before and after reaching the laboratory, has been described elsewhere (2).

Cultural Methods

Some preliminary work was necessary to determine the best medium for growth and the best method of enumerating the bacteria on the surface of bacon. Many of the types found in pickle must be present on the sides when

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[2] *Bacteriologist, Food Storage and Transport Investigations.*

removed from cure. While conditions may not be favourable for the development of all types present, some are able to grow as shown by the production of slime.

It was found that bacon infusion agars yielded slightly higher counts than beef extract agars, but the difficulty in preparing a clear medium, and the fact that the infusions contained varying amounts of salt, precluded their use as a routine medium. Beef extract, peptone agars were therefore used throughout the investigation.

To determine the optimum salt concentration, samples taken from cured bacon were diluted in brine containing from 0 to 10% salt, cultured on beef extract agar containing the same concentration of salt, and incubated at 20° C. The highest counts were obtained in the range 3 to 6% salt, the numbers falling off rather sharply at higher and lower concentrations. Within this range the maximum usually occurred on 4 to 5% salt. Since there is some advantage in using the lowest salt concentration compatible with good growth, a concentration of 4% salt was chosen with dilution in brine of the same concentration. It is of interest to note that the salt concentration of the bacon is usually about 4 to 5% (3). Consequently, it is evident that the conditions on bacon favour the development of organisms of intermediate salt tolerance.

Occasionally, counts equal to, or even higher than, those on 4% salt were obtained on 10% salt agar. It is conceivable that organisms from pickle, which prefer or are capable of growing at higher concentrations, sometimes persist for a time on the product.

Sampling

Three methods of taking the samples for surface bacterial counts were studied. The first of these, described by Haines (7), consists of outlining a definite area with sterile borers, and then removing a thin layer of the surface meat, or, in the case of ribs, the pleural membrane. Stainless steel borers, cutting a 3 sq. cm. circle, were used in this work. The second method, described by Garrard and Lochhead (4), consists of pressing a piece of filter paper of known area against the surface of the meat for 20 to 30 sec. The third was a swab method, with a piece of sheet aluminium having a 1 × 2 cm. opening used as a mask. In practice the mask was placed against the meat and the exposed area swabbed vigorously with two pledgets of absorbent cotton. The pieces of excised tissue, filter paper, or cotton obtained were in each instance transferred to dilution blanks containing coarse silica sand and shaken vigorously 100 times.

These methods were compared by taking duplicate samples by each method from the 3rd, 5th, 7th, 9th, and 11th ribs of a side of bacon. The position on the rib, from which each of the individual samples was taken, was determined by preparing a random sampling chart. The results were subjected to an analysis of variance which appears in Table I. The mean counts by the three methods show that the numbers obtained by the Haines and swab

techniques are in close agreement on the average, while that by the filter paper procedure is significantly lower. The mean square between duplicates shows that the precision decreases in the order: filter paper impression, Haines, and swab techniques. However, on the small number of observations made, only the swab technique can be said to be significantly less precise than the other methods.

TABLE I

ANALYSIS OF VARIANCE OF COUNTS ON BACON BY THREE METHODS

Method	Log mean no./cm.²	Variance between duplicates		Variance between ribs		*F*
		D.f.	Mean sq.	D.f.	Mean sq.	
Haines	5.41	5	.028	4	.086	3.07
Filter paper	4.98	5	.013	4	.240	18.46**
Swab	5.40	5	.138	4	.041	0.30

** *Exceeds 1% level of significance.*

The filter paper impression method alone showed a significant difference between the numbers of bacteria on different ribs. Since the other two sampling procedures yielded similar results and showed no differences between ribs, it is concluded that the filter paper method, although precise, gives a less accurate estimate of the actual number of bacteria present. Examination of the detailed results showed that on an arithmetical basis the filter paper method gave about 75% of the count obtained by the Haines method on the 3rd rib and only 15% on the 11th rib. Since a similar gradient was not observed by the other methods on this particular side, and as the rear ribs are usually drier than those nearest the shoulder, it appears that the filter paper method may be affected by moisture conditions on the surface.

This effect of moisture was also observed for cut surfaces of meat. Pieces of filter paper of sufficient size were used to permit a sample to be taken afterwards from the same area by the Haines technique. On somewhat dry surfaces only about 10% of the organisms were removed by the filter paper when compared with the total number removed by the combined methods. On moister surfaces, up to 30% of the organisms could be removed by the filter paper.

The criticism may be made that on meat surfaces the Haines method may also include organisms from the sub-surface layers. However, similar results were obtained on rib surfaces where only the pleural membrane is removed. Since this membrane is not disintegrated when shaken with sand, only surface organisms would be removed.

The swab method is the least precise of the three. It should be mentioned that only after considerable experience with it could the high counts reported be attained. The Haines technique was therefore adopted in this study. Although it has the disadvantage of disfiguring the sides, it gives the best estimate of the actual number of organisms present, and in practice is probably the simplest to use.

Routine Procedure

In the examination of the sides from the various plants all counts were made on the pleural membrane, since this surface has the least opportunity of outside contamination, and slime generally appears there first. Areas of 6 sq. cm. were removed from the pleura over the 2nd and 3rd ribs and over the 9th and 10th ribs, and 3 sq. cm. from the 5th rib. The 5th and 6th ribs were then removed without contamination of the pleura and stored at 1.1° C. in an atmosphere of about 95% relative humidity (over saturated zinc sulphate solution) for 15 to 17 days. The sides were rebaled and stored at 1.1° C. for 10 to 12 days as previously described (2).

At the second sampling 6 sq. cm. samples were taken from all ribs. In all cases dilutions were made in 4% brine and cultured on 4% salt agar. Counts were made after 7 days' incubation at 20° C.

Quantitative Results

Statistical methods (8) have been used to interpret some of the data. Since there was a wide variation in the number of bacteria found, the numbers were converted to logarithms both for convenience and valid statistical analysis (1).

The logarithms of the mean, maximum, and minimum number of organisms per sq. cm. are shown in Table II. From the means it may be seen that there was a slight decrease in bacterial numbers on the pleura from the anterior to posterior ribs both before and after storage. At both samplings the counts for the anterior ribs showed the greatest variation. Since the 5th rib was stored for a longer period, the second sampling from it cannot be compared with the others.

TABLE II

BACTERIAL COUNT ON PLEURAL MEMBRANE OF WILTSHIRE SIDES

(Logarithm of number of organisms per sq. cm.)

	Ribs 2 and 3		Ribs 9 and 10		Rib 5	
	Received	Stored 10–12 days	Received	Stored 10–12 days	Received	Stored 15–17 days
Mean	4.54	6.03	4.21	5.18	4.51	6.94
Maximum	6.27	8.55	5.64	7.41	6.36	9.25
Minimum	2.97	3.48	2.90	3.19	3.42	4.21
Stand. deviation	.67	1.19	.55	.93	.60	1.16
Coeff. of variation	*14.8*	*19.8*	*13.0*	*18.0*	*13.3*	*16.7*

The maximum and minimum values, the standard deviation, and the coefficient of variation (Table II) give some indication of the variation that occurs between the different sides and also between ribs. Since the standard deviation decreases with the decrease in mean number present, the coefficient of variation is fairly constant.

Sources of Variability

The variability shown by the standard deviation in Table II may be divided into that arising from three main sources: (i) the error of diluting, plating, and counting; (ii) differences in the bacterial load of sides from the same plant; and (iii) differences in the product from different plants. The variance due to these three sources is shown in Table III. For all three positions on the side the variance due to differences between sides from different plants is significantly greater than that due to differences between sides from the same plant, which in turn is significantly greater than the experimental error. The greatest source of variability is therefore between plants.

Appearance of Slime

A side may be said to be slimy when the bacteria have increased sufficiently to be visible or tactile. Although not of serious practical importance unless very pronounced, slime is considered to be an indication of improper handling at some stage during the history of the product. If the numbers are sufficient to be discerned, it is probable that taint will soon be detected.

Many of the 5th and 6th ribs that had been stored at 95% relative humidity for 15 to 17 days showed slime, and furnished an opportunity of determining the number of organisms necessary for a visible growth. On arranging the values for slimy and non-slimy sides (Fig. 1), it was found that there was a region in which sides having the same count may or may not appear slimy. Pleural membranes having a count of 5,000,000 per sq. cm. (log 6.70) or less were never slimy; with a count of 93,000,000 per sq. cm. (log 7.97) or over, the pleura was always slimy. Between these limits there were nine slimy and nine non-slimy ribs having an average count of 16,500,000 organisms per sq. cm. (log 7.22 ± 0.093). It is therefore probable that any side having a count of 16 to 17 million organisms per sq. cm. will be slimy. The counts obtained on the sides stored for a shorter time confirmed these results (Fig. 2). Haines (6) has found that slime becomes visible on stored beef at a similar value (log 7.5).

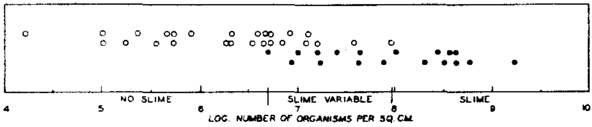

FIG. 1. *Relation between number of organisms on pleural membrane of Wiltshire sides and appearance of slime.*

In this study a visible growth was used as the criterion of slime. In practice a side may also be considered slimy if it feels slippery or if a growth can be detected by running the thumb nail over the surface. These subjective methods of detecting slime are doubtless liable to considerable variability,

TABLE III

Analysis of variance of counts on bacon from 22 plants

Variance	Ribs 2 and 3				Ribs 9 and 10				Rib 5			
	Received		Stored		Received		Stored		Received		Stored	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Analytical error	86	.0040	82	.0084	87	.0016	85	.0119	86	.0030	86	.0022
Between sides from same plant	22	.4671**	21	1.5617**	22	.2553**	22	1.0472**	22	.3641**	22	1.4774**
Between sides from different plants	21	2.2882**	20	7.0647**	21	1.5696**	21	4.1791**	21	1.8034**	21	6.6714**
Differential change with time between plants	20	2.507**			21	1.816**			21	2.339**		
Average change with time over all plants	1	127.545**			1	63.445**			1	397.096**		

**Exceeds mean square error or intra-plant variance, respectively, 1% level of significance.

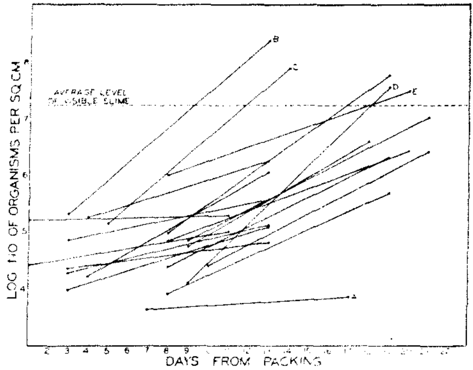

FIG. 2. *Relation between number of organisms on pleural membrane of Wiltshire sides and age from packing. (Sides B, C, D, and E showed definite slime.)*

even within a given method. Consequently, variations in the number of organisms present when a side is considered to have become slimy are to be expected. This may account for all or part of the observed variability. However, part of the variation may be real, since the visibility of equal numbers of organisms may be affected by the type of bacteria present, or by the conditions of growth on a particular side.

The significant interaction (differential change with time between plants, Table III) indicated that the bacteria do not increase at the same rate on sides from different plants. This is apparent from the slopes of the lines in Fig. 2 and suggests that the flora of different plants varied. It would appear that the flora of the sides from plant A is quite different from that of sides from plant B. On the other hand, sides from plants B and C, and possibly D, seem to have a similar flora, which grows very rapidly and is soon apparent as slime. Reports on the conditions of sides arriving in England showed that the product from plant B had a higher percentage of slime than that from the others. The conclusion seems justified that, while the majority of plants apparently have very similar types of organisms present on the sides, in a few instances quite different types may be present.

Relation of Number of Bacteria Present to Time

A significant correlation coefficient indicated that there was a relation between the number of bacteria on the sides at successive samplings and the time from the end of cure to each sampling (Table IV). The bacterial count was also correlated with the number of days from the beginning of cure and from packing.

On plotting the data (Fig. 2), this relation was confirmed and another demonstrated. Since the beginning and end of each line in Fig. 2 represent the number of bacteria present at the first and second samplings respectively, the slope of the line indicates the growth rate during this time. It can be seen that, if plants such as A, B, and C are excluded, the growth rate on sides sampled for the first time 2 to 4 days from packing is much less than on sides first sampled 8 to 10 days from packing. Although the lines are based on only two determinations, it is probably safe to conclude that under the conditions of storage there is a period of about 10 to 12 days during which the bacteria develop very slowly on the sides. After this time, development is much more rapid.

Since the appearance of slime depends on the number of bacteria present, it is evident that all sides will eventually become slimy. Apart from those having "abnormal" floras, the original load is important in determining how long the side remains free of slime. Sides having a high initial load (plant E) become slimy before those having a low initial load. From the evidence presented it may be said that, in general, if a side has a count of 100,000 per sq. cm. (about log 5) or less when packed, it will remain free of slime for 20 to 25 days under the usual conditions of transport.

TABLE IV

CORRELATION BETWEEN NUMBER OF BACTERIA ON PRODUCT, NUMBER IN PICKLE, AND TIME

Quantities correlated			Correlation coefficients (r)		
			Ribs 2 and 3	Rib 5	Ribs 9 and 10
Log of no. of organisms per sq. cm. on each side regardless of origin (total 40 sides)	*and*	*Days to each sampling from end of cure*	.60**	.80**	.56**
		Days to each sampling from packing	.58**	.80**	.57**
Log mean no. of organisms per sq. cm. on 2 sides originating from each of 20 plants	*and*	Days to each sampling from end of cure	.64**		
		Days to each sampling from packing	.61**		
		Days start of cure to each sampling	.63**		
Log mean no. of organisms per sq. cm. on 2 sides from each of 20 plants (1st sampling)	*and*	No. of bacteria per ml. in curing pickle at end of cure (4% salt agar)	.32†		
		(10% salt agar)	.25†		
		(Nut. agar 20° C.)	.28†		
		(Nut. agar 37° C.)	.14†		

** *Exceeds 1% level of significance, 38 degrees of freedom, r of .42 necessary for 1% level of significance for 35 D.f.*

† *15 degrees of freedom, r of .48 necessary for 5% level of significance.*

Relation of Number of Bacteria on Product to Number in Curing Pickle

The organisms found on bacon may come from a number of sources. Garrard and Lochhead (4) point out that the contamination on the sides prior to cure probably plays an important role. It is also possible that the organisms in curing pickle may play a part and the variation found in the number of bacteria in curing pickles (5) might be reflected in the number found on the product. To test this assumption the correlation coefficients shown in Table IV were computed. The number of bacteria on the bacon was not significantly correlated with the number of bacteria in the pickle in which it had been cured. However, the bacon examined varied in its age from cure and had been subjected to the various wiping and packing practices, and shipping conditions followed in the different plants. These factors, among others, are probably more important than the number of organisms in the pickle in determining the number of bacteria on the cured bacon.

Acknowledgment

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CANADIAN WILTSHIRE BACON

VII. SPECIFICATION OF COLOUR AND COLOUR STABILITY[1]

BY C. A. WINKLER[2] AND J. W. HOPKINS[3]

Abstract

A method of specifying the colour of bacon by measurement of its red, green, and blue spectral components is described.

Statistical evidence, based on a total of 792 observations, is presented which indicates that although the variation of these three components of colour from sample to sample of meat is to some extent correlated, each also exhibits a significant element of independent fluctuation. It is accordingly concluded that for investigational purposes, all three components should be included in forthcoming studies of colour and colour stability, and factors influencing them, in an extended series of samples. The possibility that for routine operations such as plant control a more limited analysis might suffice, will, however, also be made the subject of inquiry.

Introduction

Owing to the economic importance of the colour and colour stability of bacon exported from Canada, a study of these properties was included in a recent investigation (1) of some of the factors influencing Wiltshire bacon quality.

By visual inspection alone, it is possible to recognize considerable variation in the colour of different bacon samples, even when drawn from a single packing plant. In this case, the observed variation is probably due mainly to inherent differences in carcasses prior to curing. With product from different plants, the variability may be expected to be enhanced by differences in the curing practices employed. In these circumstances, it may be inferred that for an extensive study of the colour of bacon from various sources, a simple method of colour measurement, adaptable to routine procedure, is both adequate and desirable. A method by which only the red, green, and blue components of the colour are determined (5) was accordingly adopted.

Samples of meat may differ from one another in respect of either or both of two attributes of colour, namely chroma or colour quality, and total intensity or brightness. Variations in the former arise from differential reflection of one or more of the incident wave-bands by individual samples. Brightness,

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on the other hand, is determined by the ability of the meat to reflect all components present in the incident light. Actually, the analysis of samples with respect to colour quality is believed to be the more important, as hue rather than intensity of colour seems to be primarily responsible for the observed variation in the initial appearance of different sides, as well as for the grey or brown discolorations sometimes developing on exposure to air.

In this investigation the percentage reflection of each colour component, relative to that of a white reference standard, provided information respecting colour quality. Total reflection was not measured specifically, but the sum of the percentage reflections for the three components yielded some indication of brightness. Certain complications must however be recognized in the relation of this index of brightness to visual appearance. Most artificial light sources, including that used in this study, are much richer in the red component than in the green or blue. As bacon is likewise predominantly red, much more of the red than of either the green or blue components is reflected from the meat surface. This combination of low incidence of green and blue light with the low reflecting power of bacon for these colours necessitated the use of much more intense illumination in the measurement of the percentage reflection of these than was required in the case of the red component. In fact, in order to obtain comparable degrees of precision, each of the three colour bands was studied at a different intensity of white light. This does not affect the estimation of the percentage reflection of each component, which is independent of the incident intensity, but does suggest that the summation of the three percentage values on equal terms may exaggerate the effect of differences in the blue and green components on the visual appearance of samples, even in white light.

Experimental

The colour measurements to be considered in this connection were made on samples from 44 Wiltshire sides, comprising two sides from each of 22 Canadian packing plants. Three samplings were made of each side, by methods described in the introductory paper of this series (1). On each such occasion two observations, namely of initial colour at the time of sampling, and of colour stability as indicated by the changes after exposure for 20 hr. at 10° C. and 95% relative humidity, were made on each sample, also by procedures previously outlined (5). There were thus 792 observations in all.

Chroma or Colour Quality

In earlier publications (5, 6), colour quality was defined in terms of intensity ratios, namely, red : green, red : blue, and green : blue. Statistical analysis of this property is, however, more simply accomplished by a consideration of the variance and covariance of the component intensities themselves, rather than of their ratios, comparisons of the latter being complicated by the fact that the ratios secured are not independent of the general intensity

level. This procedure will accordingly be adopted in the subsequent papers of this series.

It was not known *a priori* whether the measurement of all three of these components was actually necessary in order to specify the colour and colour stability of bacon. This would be the case if all three components were to vary to some extent at least independently from sample to sample of meat. On the other hand, if the fluctuations in the three components, although not necessarily of the same absolute magnitude, are nevertheless closely correlated, measurement of one component alone would suffice to specify all of them. Alternatively, an intermediate situation may be envisaged in which two of the colour components are closely associated, but the third varies semi-independently. As a first step in the examination of this question, the data accumulated were used to compute the coefficients of correlation between the red, green, and blue intensities shown in Table I.

TABLE I

COEFFICIENTS OF CORRELATION BETWEEN COMPONENT INTENSITIES OF COLOUR OF INDIVIDUAL SAMPLES

Quantities correlated	First sampling	Second sampling	Third sampling	All samplings
Initial intensity				
Red × green	+.43	+.77	+.81	+.68
Red × blue	+.56	+.42	+.53	+.51
Green × blue	+.61	+.82	+.87	+.80
Stability				
Red × green	+.05	+.20	+.92	+.85
Red × blue	−.04	+.27	+.90	+.80
Green × blue	+.57	+.72	+.94	+.91
5% point	±.30	±.30	±.30	±.16

Considering first the coefficients for initial intensity at each time of sampling, given in the upper portion of Table I, all 12 of these are statistically significant, indicating that there is some real association between the component intensities of any particular sample. It is to be observed, however, that the degree of association between both red and green, and red and blue, is in no instance very high, and when the measurements for all samplings are considered collectively, is indeed quite moderate. Determination of red intensity alone consequently would not provide a highly accurate index of either the green or blue observed in the same sample, and vice versa. The association between green and blue is, however, appreciably stronger, whether the three samplings are considered individually or collectively.

In considering the observations of colour stability, or more correctly, instability, which is the property actually measured, it requires to be noted that the changes in colour on exposure after smoking (third sampling) were

much greater than those observed on either of the other two occasions, and consequently dominate the results when these are considered collectively. For this reason, in spite of the insignificant correlation between red and green and red and blue at the first two samplings, the results as a whole indicate an association between the changes in intensity which is moderately high in the case of red and green and red and blue, and quite high in the case of green and blue.

The question next arises as to whether the residual variance not accounted for by the foregoing correlations may be regarded as arising solely from experimental errors, or whether there are genuine independent fluctuations in the individual intensities from sample to sample. In the absence of any direct estimate of experimental error (single determinations only having been made on each sample) it is necessary to bring other evidence to bear on this point. This was done in two stages as follows:

The measurements of initial red, green, and blue intensity at all sampling times (396 observations) were first subjected to an analysis of variance (2) in which the main components of variation, arising from average differences between sides, between the intensity of the three colours, between the three sampling times, and the first-order interactions of these, were segregated from the second-order interactions of sides, sampling times, and individual colour intensities. As is pointed out by Fisher (3, sec. 41), such second-order interactions may be expected to be as a rule unimportant, and the variance apparently ascribable to them may accordingly be used to provide an estimate of experimental error, which will, however, be subject to inflation in proportion to the magnitude of any real interaction effects included in it.

When this was done, it was found that the residual variance of both green and blue, after correlation with red, was significantly greater than the foregoing interaction mean square, as judged by the usual variance ratio test (4). It would seem, therefore, that detectable variations in the intensity of blue and green, independent of red, may occur. The residual variance of blue after correlation with green did not exceed the interaction mean square, but owing to the possible inclusion of components other than error in the latter, this result is to be regarded as inconclusive, rather than definitely negative. In order to resolve this point, it is necessary to refer to computations, to be described in more detail in the next paper of this series, in which the homogeneity of the covariance of the green and blue intensity of individual samples within and between plants at each sampling time was examined in a manner described by Snedecor (4, sec. 12.3). By this means, significant variations in the blue intensity of samples from different plants, independent of concomitant variations in green, were demonstrable. It must be concluded, therefore, that there is in fact some element of independent variation in the intensity of each of the individual colours.

When examined in the same way, the measurements of change in colour on exposure yielded results paralleling the foregoing in that the residual variance of the change in both green and blue, after correlation with red, significantly exceeded the second-order interaction, and that the residual variance of change in blue after correlation with change in green did not. In this case, however, the subsequent analysis of covariance did not demonstrate significant differences in blue change between the product of different plants, independent of change in green.

In view of these facts, it would seem that in the investigation of an extended series of samples, measurements should be made in all three spectral regions in order to specify colour quality, particularly if it is desired to relate this to chemical or other factors. If only a limited number of samples is involved, the measurement of one component alone might suffice for comparative purposes at the present stage of development of the apparatus. For routine purposes such as plant control, it likewise seems probable that the determination of a single component might prove to be adequate, although a decision in this connection must depend on the correlation of the results of instrumental analyses with those of visual inspection. This point is currently receiving attention.

Colour Intensity or Brightness

As intimated above, the derivation of a satisfactory criterion of visual brightness from the instrumental readings made presents certain difficulties. As in the case of colour quality, it was not known beforehand whether the measurement of all three colour components was necessary, or whether one, or possibly two, of them would prove to be a satisfactory index of the total intensity. It is true that, in earlier papers, the relative brightness of different samples was assumed to be proportional to the intensity of red scatter alone, but the results then available were inadequate to demonstrate the small but significant element of independent fluctuation of each of the individual components described in the preceding section. On the other hand, in spite of this circumstance, certain considerations favour the use of the red intensity alone. One of these, touched upon already, is the probable tendency of visual inspection to assess the brightness from the intensity of the predominant red colour. Another is the fact that in addition to the independent differences noted above, there is also some degree of association between the intensities of the component colours of individual samples.

In the comparison of a limited number of samples, therefore, the red intensity may yield a satisfactory estimate of total brightness. If large numbers of samples are to be studied, as in the present investigation, the sum of the three components would seem to be preferable. A few measurements made with the apparatus without filters gave results about one-third the sum of the three percentage components obtained with filters, suggesting that the bright-

ness indicated by the latter is proportional to the intensity of reflection to be expected from a light source of the quality of a tungsten filament lamp. It is, of course, arguable that a still better index of brightness would result from a summation in which the three percentages were weighted in proportion to the absolute intensities of red, green, and blue characteristic of the incident light.

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CANADIAN WILTSHIRE BACON

VIII. COLOUR OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

By C. A. Winkler[2], J. W. Hopkins[3], and M. W. Thistle[4]

Abstract

Photoelectric measurements on two factory-cured Wiltshire sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between individual sides in respect of both total intensity and quality of colour, which would seem to have arisen mainly from differences between plants. The average range of variation between plants was: total intensity, 25%; red intensity, 23%; green, 30%; and blue, 35% of the mean. Differences in colour quality of two types, (i) due to variations in the component intensities which were correlated but not of the same absolute magnitude, and (ii) due to uncorrelated variation in the component intensities, were demonstrable.

Partial correlation studies led to the deduction of a moderate degree of association between colour quality, and pH and nitrite content, under the conditions of sampling (ii). Increased acidity was accompanied by an enhanced green and a depressed blue intensity. Increased nitrite content also tended to depress blue intensity, but apparently without significantly affecting the green. No correlation between colour and the salt, nitrate or moisture content of the meat was demonstrable.

Introduction

In this paper, some of the colour measurements made during the course of a survey of factors influencing the quality of Canadian Wiltshire bacon (1) will be discussed. As intimated in the preceding paper of this series (7), determinations were made on samples from two sides from each of 22 Canadian packing plants, or 44 sides in all. Each side was sampled, by the procedure described elsewhere (1) on three occasions, namely, (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C. The resulting 132 samples were examined in a photoelectric colour comparator (6), by means of which separate measurements were made of the intensity of the light in the blue (4,000–4,500Å), green (4,900–5,800Å), and red (5,750–7,000Å) spectral regions reflected, or more correctly, scattered, at right angles to the surface of the meat. The results secured thus fall under the two heads of total intensity or brightness, and chroma or colour quality, of which the latter is considered to be the more important for reasons already put forward (7).

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In addition to the determination of colour at the actual time of sampling, measurements were also made of colour stability, as indicated by the change in colour of each sample after exposure for 20 hr. at 10° C. and 95% relative humidity. These will form the subject of a separate communication.

Colour Intensity or Brightness

It has already been shown (7) that there is some element of independent variation in the amounts of red, green, and blue scatter from sample to sample, and that measurements of all three should consequently be used in order to specify both total intensity and quality of colour. The figures for total intensity dealt with in this section are accordingly the sums of these three components in each case, although even these, for reasons indicated elsewhere (7), are probably not entirely satisfactory indices of visual brightness.

Analysis of Variance

The variance of the 132 observations of intensity or total brightness, as defined above, was analysed, by the procedure due to Fisher (5), into three portions, ascribable (i) to average differences, over all plants, between the three sampling occasions, (ii) to average differences, over the three samplings, between sides, and (iii) to the differential variation, or interaction, of sides with sampling time. The variance between sides was further allocated within and between plants, following which a corresponding partition of the interaction variance was also made. The results of this computation are shown in Table I, while Table II summarizes some of the main features of the actual observations.

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR BRIGHTNESS

Variance due to	Degrees of freedom	Mean square
Sides	43	76.3*
Between plants	21	97.2
Within plants	22	56.3
Sampling times	2	647.1**
Interaction sides × samplings	86	47.1
Between plants	42	55.0
Within plants (residual)	44	39.6

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

The analysis of variance indicates a definitely significant difference between sampling times in the average measurements for all 44 sides, due, however, almost entirely to the increased reflectivity of the samples taken after smoking (sampling (iii)). Moderately significant differences in the average bright-

TABLE II

SUMMARY OF MEASUREMENTS OF COLOUR INTENSITY OR BRIGHTNESS

Quantity	Sampling time		
	(i)	(ii)	(iii)
Maximum			
Individual side	104.5	102.5	110.7
Plant (av. 2 sides)	96.2	97.4	108.6
Minimum			
Individual side	74.8	74.2	72.9
Plant (av. 2 sides)	79.4	75.4	78.2
Mean	87.8	88.1	94.6

ness over all three samplings of individual sides are also demonstrable. These would seem to have arisen mainly from differences between plants, as although the number of observations available is not sufficient to establish the statistical reality of the difference in the inter- and intra-plant variance, the variance between sides drawn from the same plant does not significantly exceed the residual. This is a point of some interest, since the variability of sides from the same plant probably arises mainly from inherent differences in carcasses prior to curing, whereas between plants, differences in curing practice may be operative. On the other hand, there seems to have been no pronounced differential effect of smoking on the total colour intensity of the product from different plants, the interaction of plants × samplings (Table I), although suggestive, failing significantly to exceed the residual variance.

The means for the three samplings, shown in Table II, all fall approximately midway between the respective maximum and minimum plant averages, indicating that the values for the 22 individual plants were reasonably symmetrically distributed over the observed range of variation, without any marked bias in either direction. The average range between the maximum and minimum plant averages in the three samplings was 25% of the mean. Although doubtless susceptible of some improvement, this may perhaps be regarded as not unreasonable in view of the fact that the averages were, after all, deduced from only two sides from each plant, and further that the product of different plants was of varying age when received at the laboratory.

Correlation with Chemical Analyses

In order to ascertain whether there was any readily demonstrable association between total intensity of colour and chemical composition as determined by Cook *et al.* (2, 3), a number of simple correlation coefficients were calculated. With one exception, these proved to be uniformly insignificant. The results for sampling (iii), i.e., after smoking, yielded a coefficient of $r = -0.38$ between colour intensity and nitrite concentration in p.p.m. (5% point, $r = \pm 0.30$), but the coefficients for both the earlier samplings were insig-

nificant. Nitrate and moisture content also gave insignificant coefficients for all three samplings. Salt concentration and pH were examined only for the first two samplings. Neither was correlated with intensity. The partial correlation between intensity and pH, after allowing for associated variations in nitrite concentration, was likewise insignificant for both samplings (i) and (ii).

Chroma or Colour Quality

Analysis of Variance and Covariance of Component Intensities

Table III gives the results of an analysis of variance of the component red, green, and blue intensities of the individual samples paralleling that for total brightness. The previously noted difference in total intensity between sampling times is seen to be the result of variation in all three components. Table IV shows, however, that the increase after smoking was greatest in the blue region of the spectrum and least in the red. The analysis of variance likewise indicates that the observed differences between sides in respect of red scatter were no greater than would be expected from the residual variance in this respect of successive samples from the same side, which latter, however, is sensibly greater than the corresponding residual variance of either green or blue scatter. On the other hand, the differences between sides in both green and blue are quite significant, and the inter-plant variance of blue demonstrably exceeds the intra-plant. It is also to be noted that the increase in intensity of green and blue after smoking was not of the same magnitude in the product from all plants, the interaction of plants × samplings (Table III) being significant in both instances. A complicating factor must be noted here, however, in that the sides from the eastern and western plants were smoked on different occasions, and apparently under somewhat different conditions.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	10.6	8.3**	13.8**
Between plants	21	11.3	10.6	24.0
Within plants	22	9.7	6.0	4.1
Sampling times	2	34.9*	68.7**	122.4**
Interaction sides × samplings	86	10.2	5.3	5.1**
Between plants	42	9.7	6.9*	8.3**
Within plants (residual)	44	10.6	3.8	1.9

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum									
Individual side	48.6	32.7	29.2	46.6	30.1	30.0	44.4	34.0	33.3
Plant (av. of 2 sides)	41.2	31.2	26.2	43.8	29.0	29.0	43.2	32.9	32.6
Minimum									
Individual side	*30.4*	*22.1*	*20.0*	*30.2*	*22.0*	*19.0*	*32.9*	*21.9*	*20.0*
Plant (av. of 2 sides)	33.0	23.4	20.6	33.2	22.2	19.9	34.0	23.8	20.9
Mean	38.0	26.6	23.2	37.8	26.1	24.1	39.4	28.5	26.4

The means of each component intensity again fall about mid-way between the respective maximum and minimum plant averages in all three samplings. The range between plants, averaged over the three samplings, is 23% in red, 30% in green, and 35% in blue.

Reference has already been made (7) to the covariance of the component intensities from sample to sample of meat. In view of this, two types of variation in colour quality are possible: (i) within a homogeneous system, due to the fact that the increments in the component intensities associated with a given increase in total brightness, although correlated, are not of the same magnitude; and (ii) of an irregular nature, due to uncorrelated variations in the component intensities.

It was shown (7) that variations in colour quality of type (ii) were demonstrable, and in this connection an analysis of covariance was employed to establish the fact that there were significant differences in the blue intensity of the product from different plants, independent of associated variations in green, on all three sampling occasions. A further analysis of the covariance of the red and blue intensities of the six samples (from two sides at each of three sampling times) of product from each plant into portions within and between samplings (3 and 2 d.f. for each of 22 plants respectively) was also made. This indicated that there were significant differences in blue intensity, independent of red, between samplings.

These analyses also provided information respecting the effect on colour quality of the correlated, as well as the uncorrelated, variations in component intensities. Thus, the regression coefficients within plants calculated in the course of the first analysis indicated an average change of 0.67 units in blue intensity associated with unit change in green, and those within samplings determined in the second analysis, an average change of 0.31 units of blue per unit of red. It must be concluded, therefore, that variations in colour quality of both the types mentioned above occur in practice, and that those of type (ii) may be encountered either in the product of different plants, or in that of the same plant sampled before and after smoking.

Correlation of Component Intensities with Chemical Analyses

As a first step in the investigation of the relation between the component intensities of colour and the chemical properties of the meat, the simple correlation coefficients listed in Table V were determined. These indicate that above-average nitrite content was associated with below-average intensities of both green and blue. Otherwise, they are quite insignificant, with the exception of those for blue intensity and pH (sampling (ii)), and blue intensity and moisture content (all data).

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour measurement	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
sampling (i)	-.03	-.18	-.00	-.06	-.06
sampling (ii)	-.06	-.13	+.06	+.01	-.03
sampling (iii)	—	+.08	-.21	—	+.18
All data	+.05	-.05	-.05	-.02	-.12
Green intensity					
sampling (i)	-.14	-.22	-.32*	-.41	-.12
sampling (ii)	-.13	-.19	-.09	+.17	+.02
sampling (iii)	—	-.06	-.38*	—	+.16
All data	-.18	-.11	-.28**	-.09	-.20
Blue intensity					
sampling (i)	+.01	-.02	-.14	-.26	-.25
sampling (ii)	-.15	-.21	-.20	+.40**	-.02
sampling (iii)	—	-.13	-.41**	—	+.21
All data	+.05	-.07	-.29**	+.05	-.31**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

It has to be recognized, however, that simple correlation coefficients may fail to portray adequately the relation between colour quality and chemical composition. In the first place, the former is dependent in part on the general level of intensity, owing to the differential magnitude of the correlated variations in the three spectral components. Secondly, as has been pointed out by Cook and White (4), fluctuations from sample to sample in the chemical factors themselves are not all mutually independent. Both of these circumstances may operate either to obscure or to exaggerate the real relation between individual colour components and chemical qualities. For this reason, a number of third order partial correlation coefficients have been calculated, in order to examine the relation between pairs of factors independent of associated variation in certain others. These, which are confined to the results for samplings (i) and (ii), i.e., before smoking, will be found in Table VI.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated			Independent of		Correlation coefficient	
					Sampling (i)	Sampling (ii)
Intensity		*Analysis*	*Analysis*	*Intensity*		
Red	×	salt	Nitrate,	blue and green	+.03	+.03
			Nitrite,	blue and green	−.09	+.00
			Moisture,	blue and green	+.04	−.04
			pH,	blue and green	—	+.05
Red	×	nitrate	Salt,	blue and green	−.18	−.01
			Nitrite,	blue and green	—	+.01
			Moisture,	blue and green	−.16	—
Red	×	nitrite	Salt,	blue and green	+.17	+.10
			Nitrate,	blue and green	—	+.10
			pH,	blue and green	+.07	+.06
Red	×	pH	Salt,	blue and green	—	+.12
			Nitrite,	blue and green	+.10	+.08
Red	×	moisture	Salt,	blue and green	+.10	−.14
			Nitrate,	blue and green	−.00	—
Green	×	salt	Nitrate,	red and blue	−.12	−.04
			Nitrite,	red and blue	−.04	−.03
			Moisture,	red and blue	−.21	−.02
			pH,	red and blue	—	−.09
Green	×	nitrate	Salt,	red and blue	−.19	−.02
			Nitrite,	red and blue	—	−.02
			Moisture,	red and blue	−.26	—
Green	×	nitrite	Salt,	red and blue	−.27	−.00
			Nitrate,	red and blue	—	−.01
			pH,	red and blue	−.17	+.18
Green	×	pH	Salt,	red and blue	—	−.28
			Nitrite,	red and blue	−.21	−.30
Green	×	moisture	Salt,	red and blue	−.10	+.15
			Nitrate,	red and blue	−.11	—
Blue	×	salt	Nitrate,	red and green	+.07	−.07
			Nitrite,	red and green	+.13	−.01
			Moisture,	red and green	−.02	−.12
			pH,	red and green	—	+.05
Blue	×	nitrate	Salt,	red and green	+.18	−.10
			Nitrite,	red and green	—	−.11
			Moisture,	red and green	+.10	—
Blue	×	nitrite	Salt,	red and green	−.04	−.12
			Nitrate,	red and green	—	−.15
			pH,	red and green	+.07	−.35*
Blue	×	pH	Salt,	red and green	—	+.45**
			Nitrite,	red and green	−.11	+.53**
Blue	×	moisture	Salt,	red and green	−.21	−.17
			Nitrate	red and green	−.15	—

* *Exceeds 5% point ($r = 0.31$).*
** *Exceeds 1% point ($r = 0.40$).*

They are again, for the most part, of quite negligible magnitude. Demonstrable effects are confined to the blue region of the spectrum, in which the intensity seems to be correlated negatively with nitrite content and positively with pH, i.e., the blue component increased with increasing alkalinity. There is also some indication of a negative association between green intensity and pH, but this falls short of significance. It is noteworthy that the main colour component, red, yields no indication of association with any of the chemical quantities determined.

In view of the indications obtained, the correlation of blue and green intensities with nitrite content and pH was further investigated by the calculation of still higher order partial coefficients from the results of sampling (ii). When this was done, the fourth order coefficient for green intensity and pH, independent of red intensity, blue intensity, salt content, and nitrite content, was found to be $r = -0.33$, which just exceeds the 5% point of $r = \pm 0.31$. Similarly, the fourth order partial correlation of blue intensity and pH, independent of red intensity, green intensity, salt content, and nitrite content, gave $r = 0.56$, and the fifth order partial correlation between blue intensity and nitrite content, independent of red intensity, green intensity, salt content, nitrate content, and pH, $r = -0.43$, both of which exceed the 1% point. There would thus seem to have been, under the conditions of sampling (ii) at any rate, a moderate degree of association between these two factors and colour quality, such that increasing acidity tended to raise the green and depress the blue intensity, and increasing nitrite content also tended to depress the blue intensity, but without affecting the green.

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CANADIAN WILTSHIRE BACON

IX. COLOUR STABILITY OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

BY C. A. WINKLER[2], J. W. HOPKINS[3], AND E. A. ROOKE[4]

Abstract

Photoelectric measurements on samples of two factory-cured sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between the product of individual plants in respect of the stability of total intensity of colour, and of the component intensities of red, green, and blue, on exposure of freshly-cut samples for 20 hr. at 10° C., and 95% relative humidity. Apart from a batch effect after smoking, however, there was no marked segregation of any one plant or group of plants from the remainder in respect of colour stability of product. On the average, the effect of smoking was to reduce colour stability.

Analyses of covariance demonstrated (i) a significant degree of correlation between the green and blue stability of the same sample, and (ii) a further correlation between the initial green and blue intensity and the stability of these components, samples of higher initial intensity suffering a greater reduction on exposure. Partial correlation studies suggest that increased nitrite content was accompanied by an enhanced stability of the red component of colour, but no correlation between the salt, nitrate and moisture content or pH of the meat and its colour stability was demonstrable.

In the preceding paper of this series (7), some of the colour measurements made in a survey of factors influencing the quality of Canadian Wiltshire bacon (1) were discussed. It was explained that observations were made on samples from 44 sides, two from each of 22 Canadian packing plants, each side being sampled (i) upon receipt at the laboratory; (ii) after storage for 10 days at 1° C.; and (iii) after smoking for 14 hr. at 40° C. The measurements of colour stability, now to be discussed, were secured by determining the change in intensity and quality of colour of the 132 individual samples after exposure for 20 hr. at 10° C. and 95% relative humidity. Apparatus and procedure were the same as those already described (6, 7), and the results will be dealt with under the heads previously adopted in considering the measurements of initial colour.

Change in Colour Intensity or Brightness

For reasons advanced elsewhere (6), total intensity or brightness is defined as the sum of the separate intensities of the red, green, and blue spectral components, relative to the white standard.

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Analysis of Variance

The 132 individual measurements of change in total intensity or brightness as defined above, were subjected to an analysis of variance (5), of the same form as that applied to the observations of initial colour. The results of this are given in Table I, while Table II summarizes salient features of the measurements themselves. It will be observed that the mean values in Table II are all negative in sign, indicating an average decrease in intensity of colour of the samples on exposure.

TABLE I

ANALYSIS OF VARIANCE OF CHANGE IN TOTAL INTENSITY OF COLOUR

Variance due to	Degrees of freedom	Mean square
Sides	43	39.8**
Between plants	21	71.7**
Within plants	22	9.4
Samplings	2	3064.5**
Interaction sides × samplings	86	68.9**
Between plants	42	128.3**
Within plants (residual)	44	12.2

** *Exceeds mean square residual, 1% level of significance.*

From the analysis of variance, it is to be deduced that there were significant differences in the colour stability of the product of different plants, but that the variance between sides from the same plant did not exceed the residual. On the average, stability changed significantly between samplings, but the interaction mean square indicates that the product of individual plants behaved differentially in this respect. In reality, the variance between samplings is very largely due to the decrease in average colour stability after smoking

TABLE II

SUMMARY OF MEASUREMENTS OF CHANGE IN TOTAL INTENSITY OF COLOUR

Quantity	Sampling		
	(i)	(ii)	(iii)
Maximum change			
Individual side	−12.2	−11.3	−35.7
Plant (av. 2 sides)	−10.2	− 8.0	−34.4
Minimum change			
Individual side	+ 0.1	+ 0.1	− 0.8
Plant (av. 2 sides)	− 0.1	+ 0.1	− 1.4
Mean change	− 4.7	− 1.7	−17.4

(Table II), and the differential effect is likewise largely ascribable to the fact that this decrease was confined to the sides from the eastern plants, in which it was very pronounced, and absent from those from the western plants, which were smoked in two separate batches. Unfortunately, it cannot be stated whether this discrepant behaviour was due to differences in age or other inherent properties of the meat at the time of smoking, or to variation in the conditions under which the two batches were smoked. Examination of the individual observations in samplings (i) and (ii), however, does not suggest that any one plant or group of plants stood out from the remainder in respect of colour stability of product before smoking. Rather, there was a continuous series of plant averages from the maximum to the minimum observed values.

Correlation of Change with Initial Intensity

The correlation of the observed change in total intensity of colour on exposure with the initial intensity of the freshly cut samples was also investigated, for each sampling separately. The 44 individual samples in sampling (i) gave $r = -0.28$, and those in sampling (ii) $r = -0.18$, both of which fall short of the 5% point ($r = \pm 0.30$). On the other hand, the results for sampling (iii) as a whole gave $r = +0.40$, exceeding the 1% point of $r = \pm 0.38$. This, however, was again due to the fact that the sides from the eastern plants, smoked in the first batch, exhibited both a lower average initial intensity of colour, and also an appreciably greater average decrease in intensity on exposure. So much was this the case, that when the covariance was computed about the two batch means separately, rather than about the mean of all 44 samples, a significantly negative value of $r = -0.33$ was obtained, indicating that within batches, those samples having the highest initial intensity actually tended, on the average, to show the greatest decrease on exposure.

Correlation with Chemical Analyses

Simple correlation coefficients were calculated between the change in total intensity and the salt content, nitrate content, nitrite content, moisture content, and pH of individual sides, as determined by Cook, White, and Chadderton (2, 3), but in view of the differential behaviour of the eastern and western batches after smoking, these computations were confined to the results for the first two samplings.

The resulting coefficients were all insignificant with the exception of the one for nitrite content in sampling (ii). This gave $r = +0.37$, which exceeds the 5% point of $r = 0.30$ and thus suggests that increased nitrite content may be associated with stability of colour. The corresponding value for sampling (i), $r = +0.23$, failed to attain significance, but that for sampling (ii), at $+0.39$, was practically unaffected when recomputed as a first-order partial, eliminating the effect of accompanying variations in pH.

Changes in Chroma or Colour Quality

Analysis of Variance and Covariance of Change in Component Intensities

Tables III and IV show respectively the analysis of variance and range of fluctuation of the changes on exposure, in the individual intensities of red, green, and blue. All reproduce the main features already noted in the observations of total intensity, although the differences between plants in respect of blue stability are less pronounced than those in the red and green regions. Here also the interpretation of the differential behaviour of the sides from the eastern and western plants after smoking is obscured by batch effects.

The covariance of the changes in red, green, and blue intensity in individual samples has already been discussed to some extent in an earlier paper, which contained a tabulation of the correlation coefficients bearing upon this point (6, Table I). The results for samplings (i) and (ii) agreed in indicating

TABLE III

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	7.6**	7.0**	3.5
Between plants	21	11.2**	12.9**	5.3*
Within plants	22	4.1	1.4	1.8
Samplings	2	505.2**	390.2**	175.4**
Interaction sides × samplings	86	13.2**	9.8**	5.3*
Between plants	42	23.6**	17.5**	7.9**
Within plants (residual)	44	3.3	2.5	2.9

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum decrease									
Individual side	−10.7	−3.8	−7.0	−6.7	−2.9	−3.5	−17.3	−11.7	−8.5
Plant (av. 2 sides)	− 7.6	−2.4	−4.0	−5.4	−1.4	−2.8	−15.9	−10.8	−8.2
Minimum decrease									
Individual side	− 0.4	+4.1	+2.6	+1.7	+2.5	+2.3	− 1.0	+ 1.7	+0.5
Plant (av. 2 sides)	− 0.8	+3.1	+1.2	+0.1	+2.0	+1.7	− 1.4	+ 0.9	+0.1
Mean change	− 3.8	−0.1	−0.8	−2.1	+0.5	−0.1	− 8.6	− 5.0	−3.8

a significant positive correlation between the changes in green and blue, but did not demonstrate any definite association of either of these quantities with red stability. Changes in all three intensities were significantly correlated in sampling (iii), but this must again be considered as a reflection of the batch effect mentioned above. It was also pointed out that there were significant variations in the stability of blue and green, independent of that of red, in the observations as a whole. This was, however, found to be no more pronounced in the sides from different plants than in those from the same plant, with the exception of green stability in sampling (i), which showed some variation from plant to plant independent of red.

A further analysis of the covariance of the red and green, and red and blue stability of the six samples (two sides at each of three sampling times) taken from the product of each plant, into portions within and between samplings, was also made. This paralleled a similar analysis of the actual intensities at the time of sampling, reported in the preceding paper (5). The results demonstrated (i) no significant element of correlation between the stability of the three colour components of individual samples of the bacon from the same plant, within samplings, but (ii) significant differences between samplings in the stability of both blue and green, independent of red stability. This effect was, however, confined to the samples from the eastern plants, and must accordingly be regarded as a further consequence of batch differences.

Correlation with Components of Initial Intensity

As in the case of total intensity, the correlation between the stability of red, green, and blue, and the initial intensity of these components of colour in the fresh samples was determined for each sampling.

The observations of initial red and change in red intensity in the two samplings prior to smoking gave $r = -0.21$ for sampling (i) and $r = +0.05$ for sampling (ii), both of which are statistically insignificant. For green, the values of r for the two samplings were -0.63 and -0.30. As the first of these exceeds the 1%, and the second attains the 5% point, both may be regarded as significant, and indicative of the fact that samples above average in initial green intensity tended to suffer a correspondingly greater reduction in this component of colour on exposure. A similar conclusion is to be drawn from the coefficients of -0.51 and -0.48 obtained from the measurements of blue intensity.

For sampling (iii), the observations in the red, green, and blue regions gave $r = -0.03$, $+0.36$, and $+0.50$ respectively over all samples, but these results were again clearly biased by batch differences, recomputations of the covariance about the batch means yielding $r = -0.31$, -0.42, and -0.39. Within batches therefore the indications are that, on the whole, greater decreases in each of the three component intensities occurred in those samples having the higher initial values.

Correlation with Chemical Analyses

In view of the anomalous circumstances attending sampling (iii), the correlation of the colour stability of individual sides with the results of chemical analyses was confined to samplings (i) and (ii). Even in these instances, it is necessary to take cognizance of possible complicating effects due to the association between the initial intensity of blue and green and the subsequent stability of these components, but it will be convenient first to examine the observed correlation between colour stability and the various chemical constituents studied, and then to determine whether any observed effects are to be regarded as due to the operation of the chemical factors directly upon stability, or indirectly through their influence on initial colour.

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN CHANGES IN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour change	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
Sampling (i)	+.06	+.05	−.07	−.16	−.19
Sampling (ii)	+.17	−.11	+.38*	−.09	−.06
Both samplings	+.28*	+.03	+.17	−.21*	−.32**
Green intensity					
Sampling (i)	+.19	+.10	+.36*	+.32*	+.10
Sampling (ii)	+.10	+.09	+.26	+.08	+.01
Both samplings	+.22*	+.11	+.34**	+.16	−.05
Blue intensity					
Sampling (i)	−.04	−.02	+.22	+.25	+.20
Sampling (ii)	−.02	+.06	+.25	+.04	+.32*
Both samplings	+.09	+.02	+.26*	+.09	+.05

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Of the various simple correlation coefficients shown in Table V, those of nitrite content with green and blue stability alone exhibit any measure of persistency both within and between samplings. The apparent correlation in the data as a whole between red stability on the one hand, and salt content, pH, and moisture content on the other, is not in evidence within samplings, and hence may be due to differences in the means for the two samplings, rather than to an actual causal association. There is, however, some indication of an effect of nitrite on red stability in sampling (ii). In this, as in the other instances in which a significant effect of nitrite was demonstrable, an above average concentration of this substance was associated with increased colour stability.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN STABILITY OF COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated			Independent of		Correlation coefficient	
					Sampling (i)	Sampling (ii)
Change		*Analysis*	*Analysis*	*Change*		
Red	×	salt	Nitrate,	blue and green	+.02	+.15
			Nitrite,	blue and green	+.08	+.09
			Moisture,	blue and green	−.05	+.12
			pH,	blue and green	—	+.16
Red	×	nitrate	Salt,	blue and green	+.03	−.09
			Nitrite,	blue and green	—	−.05
			Moisture,	blue and green	−.03	—
Red	×	nitrite	Salt,	blue and green	−.12	+.30
			Nitrate,	blue and green	—	+.32*
			pH,	blue and green	−.00	+.39*
Red	×	pH	Salt,	blue and green	—	−.07
			Nitrite,	blue and green	−.16	−.23
Red	×	moisture	Salt,	blue and green	−.14	−.09
			Nitrate,	blue and green	−.13	—
Green	×	salt	Nitrate,	blue and red	+.23	+.18
			Nitrite,	blue and red	+.13	+.14
			Moisture,	blue and red	+.30	+.01
			pH,	blue and red	—	+.19
Green	×	nitrate	Salt,	blue and red	+.06	+.11
			Nitrite,	blue and red	—	+.09
			Moisture,	blue and red	+.15	—
Green	×	nitrite	Salt,	blue and red	+.22	+.08
			Nitrate,	blue and red	—	+.14
			pH,	blue and red	+.22	+.10
Green	×	pH	Salt,	blue and red	—	+.11
			Nitrite,		+.09	+.03
Green	×	moisture	Salt,	blue and red	+.17	−.30
			Nitrate,	blue and red	+.07	—
Blue	×	salt	Nitrate,	red and green	−.16	−.17
			Nitrite,	red and green	−.20	−.18
			Moisture,	red and green	−.10	+.06
			pH,	red and green	—	−.17
Blue	×	nitrate	Salt,	red and green	−.04	−.02
			Nitrite,	red and green	—	+.04
			Moisture,	red and green	−.01	—
Blue	×	nitrite	Salt,	red and green	+.09	+.08
			Nitrate,	red and green	—	+.04
			pH,	red and green	−.04	+.04
Blue	×	pH	Salt,	red and green	—	−.04
			Nitrite,	red and green	+.09	−.01
Blue	×	moisture	Salt,	red and green	+.07	+.45**
			Nitrate,	red and green	+.14	—

* *Exceeds 5% point (r = 0.31).*
** *Exceeds 1% point (r = 0.40).*

As was pointed out in the previously published discussion of initial colour (5), however, simple correlation coefficients may fail to provide an adequate representation of the actual underlying relations, owing to the mutual intercorrelation, in individual samples, of (i) certain of the chemical constituents (2), and (ii) of the green and blue stability, as well as (iii) of stability and initial intensity. The analysis of these relations was accordingly pursued further by the calculation of the third order partial correlation coefficients listed in Table VI. These provide a measure of the correlation between the concentration of each chemical and the stability of each component colour intensity, after making allowance for the interrelation of both quantities under consideration with the two other component intensities of colour, as well as with one additional chemical factor.

The results now suggest that the direct effect of nitrite content on colour stability was confined to the red region of the spectrum, and that the apparent correlation of this factor with green and blue stability, noted above (Table V), was due to the interrelations mentioned. The only other significant coefficient in Table VI is that between blue stability and moisture content, independent of red stability, green stability, and salt content, in sampling (ii). Salt content itself seems to have been without measurable effect on either colour or colour stability, and pH, which appeared to have a significant influence upon initial blue intensity, likewise seems to have been without effect on the subsequent stability of this component.

The foregoing inference respecting the influence of nitrite content on red change was strengthened by the calculation of the sixth order partial correlation coefficient between these quantities, independent of salt content, nitrate content, pH, initial red intensity, green change, and blue change, which was found to be 0.43. This is the more noteworthy, since the demonstrable effects of nitrite on initial colour were confined to the blue region (5). On the other hand, the apparent association between blue stability and moisture content noted above would seem to have been spurious, as the fourth order coefficient, independent of initial blue intensity, was reduced to the insignificant level of $r = -0.09$. The absence of correlation between green change and the chemical constituents studied was confirmed by the calculation of fourth order coefficients, independent of initial green intensity, which proved to be uniformly insignificant.

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THE NITROGENOUS CONSTITUENTS OF CAT'S SUBMAXILLARY SALIVA EVOKED BY PARASYMPATHETIC AND SYMPATHETIC STIMULATION[1]

By S. A. Komarov[2] and G. W. Stavraky[3]

Abstract

The nitrogenous constituents of submaxillary saliva were studied in samples of saliva secreted by cats in response (i) to chorda tympani stimulation and (ii) to administration of adrenaline. Each of these two kinds of saliva was found to contain a different and characteristic glucoprotein. These two proteins are believed to be secreted by different cells, viz., by mucous cells in response to chorda tympani stimulation, and by serous cells in response to adrenaline. A new method is described for the determination of non-protein nitrogen in submaxillary saliva. Study of the partition of non-protein nitrogen showed that *urea represents quantitatively the main fraction of the non-protein nitrogen of* submaxillary saliva secreted in response to either parasympathetic or sympathetic stimulation. Prolonged chorda tympani stimulation causes a decrease in the permeability of the submaxillary gland to the passage of non-protein nitrogen, the fraction least affected being the urea nitrogen. The administration of adrenaline greatly increases the permeability to all fractions of non-protein nitrogen, especially urea, and this effect persists for several hours after the adrenaline administration is discontinued.

Introduction

Data concerning the protein material and non-protein nitrogenous substances in the saliva secreted by the submaxillary glands under various conditions of stimulation are very incomplete and fragmentary. As Langley (9) states in his review of the literature on salivary secretion, the chief organic material contained in the saliva from mucous (mixed) glands is mucin. Langley considered it possible that the mucin might be present in two forms, because whereas most of the mucin is precipitated by acetic acid in a stringy lump, part of it not infrequently takes the form of fine particles. He believed that some globulin is also present in the saliva of the mixed glands. The saliva from the serous glands contains globulin, or a body allied to globulin, alkali albuminate, and a small amount of serum albumin. Almost 30 years later, in a review of the same subject, Rosemann (18) was able to add very little to the above data. To quote his own words: "It seems that the protein of the saliva belongs to the class of albumins and globulins, but exact data are lacking. Similarly we know nothing about the chemical composition of the salivary mucus." Recently Kesztyüs and Martin (8) reported that the submaxillary saliva obtained on stimulation of the chorda tympani or the sympathetic nerve in the dog contains both mucin and albumin, and that the sympathetic saliva is richer in these substances than the chorda tympani saliva.

[1] *Manuscript received February 7, 1940.*
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As to the nature of the non-protein nitrogenous constituents of the saliva, the available data relate chiefly to human saliva, and largely to the mixed saliva of the mouth cavity. The following substances have been found to be normally present in the saliva: urea, ammonia, uric acid, creatinine, amino-nitrogen. Under pathological conditions, where there is retention of non-protein nitrogenous substances in the blood, the concentration of urea, ammonia, and uric acid in the saliva is increased. A review of the rather voluminous literature on the non-protein nitrogenous constituents of saliva is given by Babkin (1, 4).

The scarcity of data concerning both the nature and the concentration of protein and non-protein nitrogenous substances in the saliva obtained by stimulation of the parasympathetic or sympathetic nerves supplying the salivary glands moved us to re-investigate this problem. Recently we have been able to obtain sufficient quantities of saliva either by parasympathetic or sympathetic stimulation of the submaxillary glands in the cat to provide some data concerning both the nature of the protein material and the partition of non-protein nitrogen. We believe that the results reported here furnish some new data concerning the composition of different types of submaxillary saliva, and moreover that they warrant important conclusions as to the part played by the parasympathetic and sympathetic nerves in the activity of the submaxillary glands.

Methods

Physiological Technique

Cats, anaesthetized with nembutal, were used in the experiments. The chorda tympani and cervical sympathetic nerves were cut on both sides and the submaxillary ducts cannulated. Rhythmic stimulation was applied to the nerves for 10-minute periods interrupted by 5-minute intervals of rest. Adrenaline was administered intravenously in some experiments as a substitute for sympathetic nerve stimulation. The physiological technique was very similar to that described in the papers of Hebb and Stavraky (7) and Langstroth, McRae, and Stavraky (10, 11). For the sake of comparison, some analyses were also made of parotid saliva, which was obtained from dogs with a permanent fistula of the parotid gland.

Chemical Analysis

For the quantitative estimation of non-protein nitrogen, the proteins of the saliva were precipitated as follows: Two volumes of acetone, containing 3% acetic acid, were thoroughly mixed with one volume of saliva. The precipitate was separated by centrifuging and washed twice with a solution consisting of two parts of acetone and one part of water, and saturated with sodium chloride. This was followed by washing with 95% alcohol, absolute acetone, a mixture of three volumes of absolute alcohol and one volume of ether, and finally with ether. The original supernatant fluid and the washings were combined and evaporated to dryness under reduced pressure

at 20°C. or below. The residue was dissolved in water so that 2 cc. of the solution was equivalent to 1 cc. of saliva. In this filtrate, urea and the nitrogenous volatile bases were determined by the urease-aeration-titration method of Van Slyke and Cullen (13, pp. 547–551) and creatine + creatinine by Folin's open flask method (13, pp. 582-585). Determination of nitrogen in the whole saliva and in the protein-free filtrates was carried out by the micro-Kjeldahl procedure of Pregl (14).

Results

The Protein Constituents of the Submaxillary Saliva

In order to study the precipitability of the protein constituents of the saliva we have tried various known protein precipitants. Of special interest are the results obtained with trichloracetic acid and with acetone, which are presented in Table I. Acetic or hydrochloric acid in certain concentrations caused some precipitation in the submaxillary saliva obtained on stimulation of the chorda tympani, but the precipitation was never complete. The results obtained by precipitation of the saliva with two volumes of acetone containing 3% of acetic acid were of special interest. Not only was the precipitation of protein material always found to be complete, but the physical characteristics of the precipitates obtained from various kinds of submaxillary saliva, for example, (i) that secreted in response to chorda tympani stimulation (or to injection of parasympathomimetic drugs like pilocarpine), and (ii) that secreted in response to stimulation of the cervical sympathetic nerve (or to injection of adrenaline), were strikingly different. The appearance of these precipitates is illustrated in Fig. 1. The acetone precipitates obtained from sympathetic or adrenaline saliva were readily soluble in distilled water, dilute solutions of sodium carbonate, sodium and potassium hydroxide, and also in dilute solutions of hydrochloride acid, and formed non-

TABLE I

PRECIPITABILITY OF PROTEIN MATERIAL IN VARIOUS KINDS OF SALIVARY SECRETION BY TRICHLORACETIC ACID AND ACETONE IN THE PRESENCE OF ACETIC ACID

Saliva	Trichloracetic acid (5%, 7%, or 10%)	2 vol. acetone containing 3% acetic acid	Viscosity of alk., neutral, or acid soln. of acetone, ppt.
Parotid (dog)	Complete pptn.	Flocculent ppt.	Non-viscous
Submaxillary (cat), chorda tympani	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), pilocarpine	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), sympathetic	No ppt.	Flocculent ppt.	Non-viscous
Submaxillary (cat), adrenaline	No ppt.	Flocculent ppt.	Non-viscous

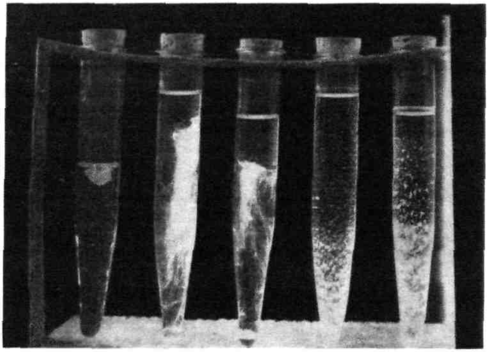

FIG. 1. *Appearance of the precipitates formed when various samples of cat's submaxillary saliva were poured into centrifuge tubes containing double the volume of 3% solution of acetic acid in acetone and the contents of each tube were thoroughly mixed by gentle rotary movements. Tubes 1 and 2, precipitates in saliva secreted in response to chorda tympani stimulation; both types—almost solid lumps or a sticky mass with numerous threads adherent to the sides—are quite typical. Tube 3, precipitate in saliva secreted in response to intravenous injection of pilocarpine; similar to that in Tube 2. Tube 4, precipitate in saliva obtained on stimulation of the cervical sympathetic nerve, and Tube 5, in saliva secreted in response to intravenous administration of adrenaline; in both, protein material was precipitated in very small and light flocculi with no tendency to adhere to the sides.*

viscous solutions. The acetone precipitates obtained from chorda tympani saliva were fairly soluble in dilute alkalis, the solutions being extremely viscous. These precipitates could also be dissolved in distilled water and in $0.5N$ hydrochloric acid, but only after many hours of vigorous stirring; the resulting solutions were very viscous.

Thus on the basis of precipitability with trichloracetic acid and with acetone it may be concluded that the parotid saliva and the parasympathetic and sympathetic submaxillary salivas have each a different protein.

A more detailed study was made of the general properties and elementary composition of the precipitates obtained by adding two volumes of acetone containing 3% of acetic acid to the submaxillary saliva secreted in response to stimulation of the chorda tympani or to injection of adrenaline. The corresponding precipitates will be referred to respectively as "Substance C" (chorda tympani saliva) and "Substance A" (adrenaline saliva).

The freshly secreted saliva was treated with acetone-acetic acid reagent immediately after collection of the sample; the precipitate was separated the following day by centrifuging, and washed twice with the same reagent diluted with one-third its volume of distilled water, twice with 95% alcohol, and finally with dry acetone and with ether. At this stage "Substance A"

was a very light, snow-white powder, readily soluble in distilled water, dilute alkalis (0.02*N* sodium hydroxide) or dilute acids (0.005*N* hydrochloric acid), the solutions being non-viscous. "Substance C" in appearance was a heavy, solid mass, which was very resistant to trituration in a mortar; in powder form it was completely soluble in 0.02*N* sodium hydroxide, but solution could be accomplished only after long and vigorous stirring. It was even more difficult to dissolve "Substance C" in distilled water. Its solubility in hydrochloric acid varied with the concentration; complete solution could be obtained only in concentrations slightly above 0.25*N*; once solution was complete, dilution with distilled water did not cause the characteristic precipitation described by Hammarsten (6) for his preparations of mucin from the submaxillary glands. All the solutions of "Substance C" (in distilled water, sodium hydroxide, or hydrochloric acid) were very viscous. Both "Substance C" and "Substance A" could be precipitated from these solutions in dilute hydrochloric acid with four volumes of absolute alcohol, "C" in the form of sticky masses (threads or lumps), "A" in the form of light flocculi. In both cases the precipitation was complete if traces of sodium chloride were added.

In this stage of preparation both "Substance A" and "Substance C" were obtained from many samples of saliva, and they showed quite consistent percentages of nitrogen content as determined by the Kjeldahl method, viz., for "Substance A", 8.8%, and for "Substance C", 8.2%. The reducing power, which was determined by the Hagedorn-Jensen method in the products of acid hydrolysis (3 hr. at 100° C. in *N* sulphuric acid), was identical for both preparations, being equivalent to a 33% glucose content.

An attempt was made to purify "Substance C" further, by the method of Hammarsten (6). For this purpose about 150 mg. of the substance was finely powdered in a mortar and dissolved in a minimal amount of water with the aid of dilute hydrochloric acid. Complete solution took place when the concentration of hydrochloric acid was increased to slightly above 0.25 *N*. When the solution was diluted with water no precipitation was observed. Therefore precipitation was effected with four volumes of alcohol and traces of sodium chloride. A heavy flocculent precipitate was formed almost instantaneously; on centrifuging it became a compact, sticky mass. This was separated by centrifuging and washed several times with alcohol in increasing concentrations, and finally with absolute alcohol, alcohol-ether (3 : 1), acetone and ether, and dried to a constant weight in a vacuum desiccator. In this form the product was just as resistant to pulverization as in previous stages. In finely powdered form it appeared as a pure white and remarkably heavy substance.

"Substance A" was purified in the same manner as "Substance C". The final product was a pure white substance which, in contrast to that from "Substance C", was very light and easily pulverizable.

The solubilities of both preparations were the same as in the first stage of purification.

The general protein colour reactions were positive both with "Substance A" and "Substance C". The proteins (0.2 and 0.4% solutions in 0.03*N* sodium hydroxide or in 0.03% hydrochloric acid) were not precipitable with tungstic or phosphotungstic acid any more than with hydrochloric, sulphuric, or acetic acid at the same pH. With phosphotungstic acid the solutions of both substances showed only a very slight opalescence at pH 1. With tungstic acid the solutions of "Substance A" remained quite clear throughout the range pH 7.0 to 1.0, whereas solutions of "Substance C" showed a slight turbidity at a faintly acid reaction to litmus and a small flocculent precipitate appeared at a point at which Congo paper turns distinctly blue; precipitation was never complete, however.

The viscosity (relative to that of water at 25° C.) was determined, by Ostwald's modification of Poiseuille's method (5, p. 71), in 0.4% solutions of Substances A and C in 0.003*N* sodium hydroxide and in 0.2% solutions in dilute hydrochloric acid. Acid solutions of both substances were prepared by dilution of the alkaline solutions with equal volumes of 0.017*N* hydrochloric acid. Both solutions remained clear. The results were as follows:

Solution	Water	0.4% alkaline		Water	0.2% acid	
		A	C		A	C
Time, sec.	80	156	372	79	82	90
Viscosity	—	1.95	4.65	—	1.04	1.14

Elementary analysis of the substances (nitrogen by the micro-Dumas method, total sulphur by the micro-Carius method) was carried out in the Laboratories of the Rockefeller Institute for Medical Research in New York, through the courtesy of Dr. P. A. Levene. Table II shows the results obtained, along with the analytical data for known preparations of mucin from the submaxillary glands for the sake of comparison.

A low percentage of nitrogen (from 8.20 to 8.89%) and a high reducing power after acid hydrolysis (equivalent to 33% of glucose) were consistently found in all the preparations isolated from both chorda tympani and adrenaline submaxillary saliva. These findings indicate that the proteins of the submaxillary saliva belong to the group of glucoproteins. This conclusion is in complete agreement with general opinion. The fact that the values were identical for both the nitrogen and the reducing power in the acetone precipitates before and after purification indicates that no other proteins are present in significant quantities in the submaxillary saliva of the cat. The fact that trichloracetic acid does not cause any precipitation in submaxillary saliva suggests an even more definite conclusion, namely, that there are no other proteins, such as globulin or albumin, for example, in submaxillary saliva besides the two glucoproteins described above.

TABLE II

ELEMENTARY ANALYSIS OF VARIOUS MUCIN PREPARATIONS

	Substance A	Substance C	Scherer*	Obolenski*	Hammarsten*
C, %	—	—	52.00	52.30	48.80
H, %	—	—	6.90	7.20	6.80
N, %	8.76† 8.80‡	8.89† 8.20‡	12.80	11.90	13.30
S, %	1.83	2.43	—	—	0.84
Ash, %	0.85	1.25	—	—	—
Reducing power as % glucose after acid hydrolysis	32.80	32.90	—	—	—

* *The data of Scherer, Obolenski, and Hammarsten are quoted from Table XIX, p. 126, "Hexosamines and mucoproteins", by P. A. Levene (Longmans, Green & Co., London, New York, etc., 1925).*

† *Dumas method.*

‡ *Kjeldahl method.*

Thus, as shown above, "Substance A" (a protein isolated from the submaxillary saliva secreted in response to adrenaline administration) and "Substance C" (a protein isolated from the submaxillary saliva secreted in response to chorda tympani stimulation) show distinct differences in their general properties, especially with regard to the physical characteristics of the precipitates and the viscosity of their solutions. They also show significant differences in their elementary composition, particularly with regard to sulphur and nitrogen content (the latter as determined by the Kjeldahl method). Therefore these two substances must be regarded as two chemically distinct glucoproteins. This conclusion is corroborated by the recent observations of Langstroth, McRae, and Stavraky (10), who found that the extinction coefficient for chorda tympani saliva, as determined by absorption spectrum measurements, and the protein-nitrogen values differ from those for adrenaline or sympathetic saliva.

Evidence has been accumulating lately that the chorda tympani and the sympathetic nerve supply different sets of cells in the submaxillary gland, the chorda tympani supplying the mucous cells and the sympathetic the serous cells (2–4, 10, 15, 17).

Thus all the experimental evidence so far available suggests that the mucous cells, which are under the control of the chorda tympani, secrete a specific glucoprotein different from the protein material secreted by the serous cells, which are under the control of the sympathetic nerve.

It is also of interest that both these proteins differ strikingly in their elementary composition from the preparations of mucin previously isolated from the submaxillary glands (6; 12, p. 126). This suggests the possibility that during the process of secretion the glandular proteins are transformed into the different types of proteins contained in the salivary secretion.

The Non-protein Nitrogen of the Submaxillary Saliva

As follows from the previous section, the proteins of the submaxillary saliva differ from those of the blood in respect of their precipitability by various reagents. From the analytical point of view, it is important that they are not precipitable either by tungstic acid or by trichloracetic acid. Obviously none of the methods based upon the use of the above reagents are suitable for the quantitative determination of non-protein nitrogen in the submaxillary saliva. After having investigated a number of other protein precipitants we arrived at the conclusion that a very convenient and exact method of separating the protein from the non-protein fraction of the submaxillary saliva is precipitation of the saliva with two volumes of acetone, containing 3% of glacial acetic acid (described under Methods). The most convincing evidence of the completeness of fractionation by this method is the fact, repeatedly observed, that both the percentage of nitrogen in the acetone precipitate and the reducing power as determined after preliminary acid hydrolysis are constant and do not change even when the product is purified in the rather complicated manner described in the previous section. A further argument in favour of the procedure in question is that hundreds of protein nitrogen determinations by this method have been made by Langstroth, McRae, and Stavraky (10, 11), the results being checked by the spectrographic method, and it was found that they agreed well. Moreover the results thus obtained were sufficiently exact to permit of mathematical interpretation, which resulted in the development of a mathematical theory of protein secretion by the submaxillary glands (11).

Protein-free filtrates obtained by the above-described acetone method from several relatively large samples of submaxillary saliva (10.5 to 23 cc.) were analysed for their content of nitrogenous volatile bases, urea, creatine bodies, and amino-nitrogen. The results of these analyses are given in Tables III and IV; the former relates to the saliva secreted in response to chorda tympani stimulation and the latter to that obtained on administration of adrenaline.

While reserving fuller discussion of the above data for the following section, we should like to emphasize certain important observations here. Urea nitrogen is undoubtedly the main component of the non-protein fraction of the submaxillary saliva, forming from 50 to 86% of the total non-protein nitrogen. Both the absolute concentration of urea nitrogen in the saliva and the relative concentration with regard to total non-protein nitrogen are markedly higher in the adrenaline saliva and in the samples obtained by chorda tympani stimulation during the after-effect of adrenaline than the corresponding values in the chorda tympani saliva. Volatile nitrogenous bases were found to be always present in the submaxillary saliva in extremely low concentrations, namely from 0.4 to 0.7 mg. per cent nitrogen. These data are in complete agreement with the observations of Schmitz (19). Under the conditions of our experiments the variations to be found in Tables III and IV are entirely within the limits of experimental error. The amino-nitrogen

TABLE III

ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI

Experiment	Sample	Stim. chorda tymp., coil cm.	Secretion		Analytical data												
			Duration, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		Unidentified N, % of NPN
								Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	
No. 1, April 15 (1 gland only)	I	17	40	2.60	42.6	32.1	10.50	5.90	56.0	0.70	6.7	0.69	6.6	0.90	8.6	22.1
	II	12	30	4.40	17.5	9.1	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.90	10.7	17.5
	III	8	30	3.80	12.3	3.9	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.82	9.7	18.5
No. 2, May 8 (2 glands)	I	17	40	0.70	71.7	51.0	20.70	12.90	62.0	0.70	3.4	1.25	6.0	1.98	9.6	19.0
	II	12	30	3.05	42.6	25.8	16.80	11.40	68.0	0.60	3.6	1.19	7.0	1.70	10.0	11.4
	III	8	30	3.17	29.1	15.1	14.00	10.40	74.0	0.50	3.6	1.11	7.9	1.55	11.0	3.5
No. 3, March 10	I	12	18 hr.	1.50	—		9.65	6.63	68.7	0.56	5.8	0.80	8.3	0.35	3.7	13.5
Composite sample from several experiments		—	—	—	—		9.50	5.10	53.6	0.45	4.4	—	—	—	—	—

TABLE IV

ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI OR ADMINISTRATION OF ADRENALINE

Experiment	Sample	Stimulation	Secretion		Analytical data												
			Duration, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		Unidentified N, Per cent of NPN
								Mg %	Per cent of NPN	Mg. %	Per cent of NPN	Per cent Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	
No. 5 March 24	I	Chorda tympani, coil 16 cm.	40	2.90	41.4	29.7	11.7	6.20	53.0	0.50	4.3	0.56	5.0	1.3	11.1	26.6
	II	Adrenaline	80	0.50	35.8	8.7	27.1	17.50	64.6	0.50	1.8	1.43	5.3	2.2	8.1	20.2
	III	Chorda tympani, coil 12 cm.	60	1.90	47.0	19.2	27.8	21.50	77.3	0.50	1.8	1.34	4.8	1.7	6.1	10.0
No. 6 May 27	I	Chorda tympani, coil 15 cm	40	3.00	37.7	28.6	9.1	5.04	55.4	0.70	7.7	0.50	5.5	0.4	4.4	27.0
	II	Adrenaline	60	0.45	61.1	38.7	22.4	13.45	60.5	0.70	3.1	1.50	7.0	0.9	4.0	25.4
	III	Chorda tympani, coil 7 cm	30	2.66	58.8	36.2	22.6	16.80	74.4	0.70	3.1	1.60	7.1	0.7	3.1	12.3
Composite sample from several experiments		Adrenaline	—	—	—	—	25.8	19.00	73.6	0.52	2.0	—	—	—	—	—

values are also low, ranging from 0.4 mg. per cent to 2.2 mg. per cent. In this respect our data agree with the observations of Updegraff and Lewis (20).

In the tables the figures arbitrarily given as representing creatine + creatinine nitrogen do not represent only these substances, since the development of the colour in most cases was attained almost at once on adding alkali to the mixture of protein-free filtrate and picric acid, and furthermore the colour did not fade as quickly as in the solutions of creatinine having the same maximum colour. The concentrations of apparent creatine + creatinine were found to be from 0.5 to 0.6 mg. per cent in typical chorda tympani saliva, and were considerably greater in the samples obtained in response to injection of adrenaline, i.e., from 1.43 to 1.5 mg. per cent, and remained high in the samples obtained on chorda tympani stimulation during the after-effect of adrenaline (viz., 1.34 to 1.6 mg. per cent).

Discussion

Considerable variations were observed in the concentrations of the various nitrogenous constituents of submaxillary saliva, depending on the conditions of stimulation. A sharp progressive fall in the concentration of protein nitrogen in the successive samples of saliva obtained by rhythmic stimulation of the chorda tympani (cf. Table III) was regularly observed, which merely confirms facts established long ago. Intravenous administration of adrenaline in massive doses interposed between two periods of chorda tympani stimulation (Table IV) resulted in an increase in the concentration of total nitrogen in the chorda tympani saliva obtained after adrenaline administration. Whereas the concentration of non-protein nitrogen in this type of saliva was always greater, the percentage of protein nitrogen varied from one experiment to another. Thus, e.g., in the experiment of March 24, it was lower in the chorda tympani saliva obtained after adrenaline administration than in that obtained before, while in the experiment of May 27 it was definitely higher. These variations might be due partly to the degree of vasoconstriction produced by massive doses of adrenaline and the necessity of applying a stronger electric current to the nerve in order to obtain an adequate volume of saliva. Furthermore Langstroth, McRae, and Stavraky (10) consider that prolonged vasoconstriction produced by adrenaline administration may be partly responsible for the increased concentration of organic colloidal material in cat's submaxillary saliva obtained by subsequent stimulation of the chorda tympani.

Another conclusion which may be drawn from these experiments concerning the protein material in the submaxillary saliva is that the total output of protein material in the submaxillary saliva in response to adrenaline stimulation is not influenced by the previous activity induced in the gland by chorda tympani stimulation, but presumably depends on the store of the parent substance in the form of granule material in the serous cells. This is evident from the fact that in two experiments (Nos. 5 and 6) the rate and the duration of secretion obtained on administration of adrenaline were practically identical

(rate 0.5 and 0.45 cc. in 10 min., duration 80 and 60 min. respectively), but the concentration of protein nitrogen differed markedly in the two samples (being 8.7 and 38.7 mg. per cent). It is important to note in this connection that the preliminary secretions of the gland in response to chorda tympani stimulation were almost similar in amount in these experiments (viz., 2.9 and 3.0 cc.), that they were obtained over the same period of time (40 min.) and that they contained practically the same amount of protein (29.7 and 28.6 mg. per cent of protein nitrogen). These facts support the theory that parasympathetic and sympathetic stimulation respectively act on different cytological elements of the submaxillary gland.

Our data on non-protein nitrogen and its various fractions permit some conclusions to be drawn concerning changes in the "permeability" of the submaxillary glands to these substances under various conditions of stimulation. It was found practical for the purpose to classify the various fractions of non-protein nitrogen into three main groups:

(1) Urea nitrogen (represented in Figs. 2 and 3 by the symbol "UrN").

(2) "Creatine bodies fraction" + "volatile bases fraction" + amino-nitrogen ("RN").

(3) Unidentified nitrogen ("XN").

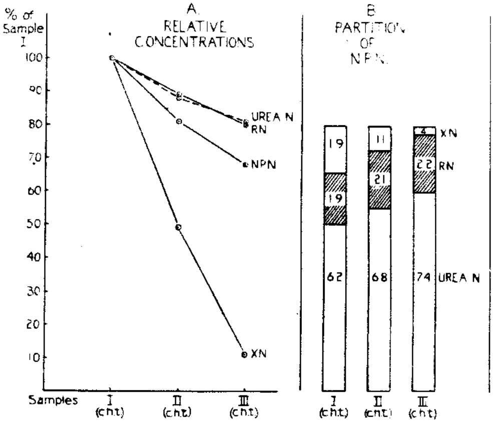

FIG. 2. *Experiment 2. Influence of chorda tympani stimulation on the non-protein nitrogen of submaxillary saliva.*

Samples I, II, and III are successive samples. (For full data see Table III.) Section A, values expressed as percentages of values for the first sample. Section B, values for three main fractions of non-protein nitrogen expressed as percentages of non-protein nitrogen. NPN, *non-protein nitrogen;* RN, *combined nitrogen of the "creatine bodies" + volatile bases + amino-nitrogen;* XN, *unidentified nitrogen.*

Although in different animals the initial values both for total non-protein nitrogen and for its fractions varied, each experiment provided convincing evidence that the concentration of certain moieties in the saliva underwent marked changes under various experimental conditions. The most probable explanation of this phenomenon is that the changes in the composition of the saliva occurred as a result of altered permeability of the cellular membranes.

Fig. 2 represents the results of two experiments in which the chorda tympani was stimulated. Section A shows changes in the concentrations of the three main fractions, expressed as percentages of the values for the first sample; Section B shows the relative concentrations of the same three fractions as percentages of the total non-protein nitrogen. In both experiments qualitatively identical results were obtained: the concentrations of total non-protein nitrogen and of each of the main fractions are lower in each successive sample; the relative concentration of "unidentified nitrogen" decreases in the successive samples to a much greater extent than that of the other fractions. Section B shows how these processes affect the partition of non-protein nitrogen in each consecutive sample. It will be seen that, as the glands secrete under the influence of the chorda tympani, an increasingly greater part of the non-protein nitrogen is formed by urea, while the fraction of the creatine bodies + volatile bases remains virtually stationary and the percentage of "unidentified nitrogen" falls considerably (especially in Experiment 2). The obvious conclusion is that continuous stimulation of the chorda tympani for several hours causes an appreciable decrease in the glandular permeability, which though it affects but slightly the passage of the small molecules such as urea (and perhaps "RN"), inhibits considerably the passage of the larger molecules such as "unidentified nitrogenous substances". This conclusion is in agreement with the results of Langstroth, McRae, and Stavraky (10), who observed a gradual decrease in the concentrations of the total anions and non-protein nitrogen in the saliva secreted under chorda tympani stimulation and ascribed this phenomenon to decrease of the glandular permeability.

Fig. 3 shows the results of Experiments 5 and 6, in which between two periods of prolonged chorda tympani stimulation (Samples I and III), the submaxillary gland was subjected for 80 and 60 min. respectively to stimulation by repeated injections of massive doses of adrenaline (Sample II). In both experiments the absolute concentration of every fraction of non-protein nitrogen was increased in the samples of adrenaline saliva, but in various degrees. In Experiments 5 and 6 respectively the concentration of non-protein nitrogen rose to 232 and 240%, that of urea nitrogen to 282 and 267%, while "RN" was 175 and 194%, and unidentified nitrogen 168 and 238%, of the initial values. After the administration of adrenaline was discontinued and chorda tympani stimulation was recommenced, the urea nitrogen continued to rise, reaching 347 and 333%, the "RN" fell slightly to 150 and 188%, while the unidentified nitrogen returned practically to the

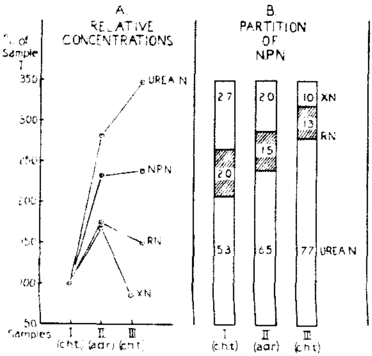

FIG. 3. *Experiment 5. Effect of adrenaline on the non-protein nitrogen of submaxillary saliva.*

Samples I (ch. t.), II (adr.), III (ch. t.) were obtained by chorda tympani stimulation, adrenaline administration, and chorda tympani stimulation immediately after discontinuing adrenaline. (See also Table IV.) Explanation of symbols as in Fig. 2.

initial values, being 85 and 114%, and the non-protein nitrogen remained stationary. Thus the administration of adrenaline increased the permeability of the gland to all fractions of non-protein nitrogen and especially to the passage of urea. The greater permeability to urea persisted or even continued to increase further for a considerable time after the administration of adrenaline was stopped and chorda tympani stimulation substituted, but the permeability to unidentified nitrogen soon returned to normal. The final result of all these phenomena was a steady and marked increase in the percentage of urea nitrogen and progressive decrease in the percentage of unidentified non-protein nitrogen, with the percentage of "RN" remaining practically stationary. The above-reported facts supplement the results of Langstroth, McRae, and Stavraky (10), who found that samples of adrenaline saliva are richer in inorganic salts, and samples of chorda tympani saliva obtained after adrenaline administration are richer in inorganic salts and glucose than samples of chorda saliva obtained before adrenaline administration. They attributed these results to the increased permeability of the glandular membrane which persists for several hours after adrenaline administration.

Acknowledgment

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CANADIAN WILTSHIRE BACON

X. DISTRIBUTION OF CHLORIDE[1]

BY W. H. WHITE[2] AND W. H. COOK[2]

Abstract

Highly significant differences in chloride content were observed at different positions across the ham of Wiltshire sides, the portion next to the tank pickle during cure usually containing the most chloride. Of the various factors studied, the number of injections used for pumping a side, and the age from cure, were the only ones found to affect the chloride distribution. Greater uniformity was obtained by increasing the number of injections, and with increasing age from cure. Within the range used in practice for curing Wiltshire sides, the chloride content of the pickles or the number of days in cure was not related to the chloride distribution. An equation is given relating the uniformity of chloride distribution to the number of injections used per side in pumping, and the number of days from the end of cure. This equation indicates that, on the average, the variations in the chloride content within the ham of a Wiltshire side will be reduced to the same magnitude as the variations between sides cured in the same plant, after holding at 1.1° C. for periods of about 12, 26, and 30 days, for sides receiving 27, 8, and 0 injections, respectively.

Introduction

Curing of bacon involves not only the addition of a sufficient quantity of curing salts, but also their distribution throughout the meat. The period of maturation, following removal of the bacon from cure, is believed to improve the quality of the product, particularly with respect to colour uniformity and stability, and the development of the typical bacon flavour. Because of the relatively short curing periods employed in the preparation of Wiltshire bacon, it seems probable that the distribution of salts takes place mainly after removal from cure. Presumably such distribution must reach a certain level of uniformity before the desirable changes involved in maturation can occur. The apparent importance of a uniform salt distribution, both from its direct effect on palatability and its possible indirect effect on maturation, led to a study of this subject on factory-cured Wiltshire bacon.

The content of curing salts and properties of Wiltshire bacon obtained from different Canadian packing plants, and the effect of storage and smoking on these quantities, have been reported in earlier papers of this series (5, 6). The present study was confined to the distribution of chloride throughout the ham

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at the different samplings, with a few additional determinations on the content and distribution of chloride, nitrate, and nitrite, in pumped and unpumped sides.

Since the sides obtained from different plants varied in age from two to 11 days from the end of cure when the first analyses were made, it is obvious that the results do not give a measure of the rate of salt penetration during cure. In commercial practice, moreover, pickle is injected into Wiltshire sides at a number of points before they are placed in the curing tank. This practice, termed pumping, doubtless tends to increase the total salt content and to improve the uniformity of salt distribution, but the variations between the methods employed in different plants could easily mask the effect of differential rates of salt penetration in different carcasses. Factors involved in pumping include the amount of pickle injected, and the position and number of stitches used. Of these quantities, only the effect of the number of stitches could be determined, since the position of the stitches cannot be described in quantitative terms, while the amount of pump pickle added to the sides in question was unknown. It appears, however, that the quantity of pickle retained by the sides is a function of the number of stitches employed (4).

Material, Sampling, and Methods

A description of the sides used in this study, the method of transfer from the plants to the laboratory, and the conditions of storage between samplings, has been reported in the first paper of this series (7). The samples were obtained in the conventional manner (1) from a central strip, about 1½ in. wide, taken from the slice of ham removed for analysis at each sampling (7). These strips included the cross section of the femur, since certain practical operators attach considerable importance to the salt concentration of the meat in the vicinity of the bone. There is also some evidence (3) that the adductor and vastus intermedius muscles do not absorb salt readily. Each strip was divided into three, instead of the five or more sections usually employed in studies of this sort. This practice was followed since the hams on Wiltshire sides are rather small and, being compressible, tend to vary somewhat in thickness, depending on the conditions and method of baling and transport. The position from which each of the samples was taken is shown in Fig. 1. The material from Position 1, on the inside of the ham, was adjacent to the tank pickle during cure; that from Position 2 represented the middle of the ham, surrounding the bone; while Position 3 represented the meat adjacent to the layer of fat and skin, through which little salt is likely to penetrate during cure.

The lean meat was separated from the bone, fat, and connective tissue, and each portion thoroughly mixed and ground by passing it through a food chopper. The samples were then frozen and stored at −29° C., until required for analysis. The chloride content was determined according to the official method of the A.O.A.C. (9, p. 354), in which the sample is ashed, leached with hot water, and the chloride content of this extract found by the Volhard

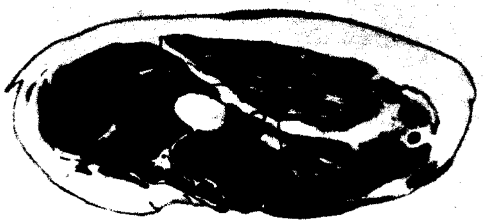

FIG. 1. *Portions of Wiltshire hams used for determining distribution of chloride.*

procedure. Statistical methods (10) were employed for interpreting the results.

The entire group of 44 sides were analysed at the first and second samplings; i.e., when received at the laboratory, and again after 10 days' storage at 1.1° C. Only 16 of these sides were analysed for chloride distribution at the third sampling, i.e., after smoking. While in subsequent tables the results for the entire group are considered together for the first two samplings, those for the 16 sides analysed three times are also considered separately, in order to provide comparable results over all three samplings.

Mean Chloride Content by Positions and Samplings

The mean age and sodium chloride content by positions and samplings over all sides are given in Table I. The chloride content of the slice of ham from which the chloride distribution samples were taken has already been reported (6). The table is divided into two main sections, the first giving the results for the 44 sides analysed twice and the second the results of the 16 sides analysed three times.

Although the mean age of the entire group of sides from cure was about seven days at the time of the first sampling, it is evident that the sodium chloride content at Position 1, next to the tank pickle, was much higher than at Positions 2 and 3. This gradient still existed, but was much smaller, at the second sampling when the sides were about 18 days old. The group of 16 sides analysed three times indicated essentially the same behaviour at the first two samplings, and also showed that the chloride is quite uniformly distributed after smoking, when the mean age for the group was about 24 days.

The results reported in the last column represent the best estimate of the true chloride content of the hams, since these figures (6) were obtained by grinding and sampling a piece of each ham several times as large as that of the entire strip used in the salt distribution studies. Other investigators (8) have observed that this central-strip sampling procedure does not represent

TABLE I

MEAN SODIUM CHLORIDE CONTENT OF SIDES BY POSITION AND SAMPLING

Sampling	Mean age of sides from cure, days	Position	Mean sodium chloride content: By position, %	By sampling, %	From previous analyses (6), %
For all 44 sides—sampled twice					
First	7.4	1 2 3	6.42 3.45 3.03	4.30	3.43
Second	17.8	1 2 3	4.49 3.78 3.46	3.91	4.42
For 16 sides—sampled three times					
First	9.9	1 2 3	6.87 3.47 2.69	4.34	3.19
Second	21.4	1 2 3	4.46 3.73 3.33	3.84	4.19
Third	24.4	1 2 3	4.06 3.90 4.01	3.99	—

the true salt content of the ham. The present results indicate that the mean chloride content over all three positions in this central strip apparently overestimates the true chloride content at the first sampling and underestimates it at the second. In more specific terms the chloride content at Position 1 was much above the average at the first sampling, while that at Positions 2 and 3 was still below the average at the second sampling. From this it appears that the positions chosen represent not only those likely to have the highest chloride content initially, but also those that are the last to be affected by the movement of chloride throughout the ham.

The significance of the observed differences in the chloride content between positions at the different samplings was determined by an analysis of the variance for each sampling, into portions attributable to: between positions; between sides from different plants; between sides from the same plant; the interaction positions × plants; and sampling and analytical error. The results, which appear in Table II, show that the difference between sides from different plants is usually significantly greater than the differences between sides from the same plant, and that this in turn usually exceeds the sampling and analytical errors. This merely confirms the findings reported

TABLE II

ANALYSIS OF VARIANCE OF SODIUM CHLORIDE CONTENT AT DIFFERENT SAMPLINGS

Variance attributable to	Sampling					
	First		Second		Third	
	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
For all 44 sides—sampled twice						
Between positions	2	149.926**	2	12.206**	—	—
Between plants	21	4.148	21	3.320**	—	—
Between sides	22	2.202**	22	0.797**	—	—
Differential effect, position × plants	42	1.977**	42	0.433**	—	—
Sampling and analytical error	44	0.674	44	0.177	—	—
For 16 sides sampled for salt distribution—three times						
Between positions	2	79.186**	2	5.262**	2	0.102
Between plants	7	8.129**	7	5.227**	7	7.329**
Between sides	8	0.798**	8	0.583	8	0.844
Differential effect, position × plants	14	1.232**	14	0.343	14	0.250
Sampling and analytical error	16	0.175	16	0.287*	16	0.434

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

in an earlier paper (6). The fact that these differences were sometimes not significant at certain samplings in the present study does not indicate disagreement with the earlier results, since they dealt with the average effect over all sides and samplings.

The present study is concerned primarily with the variance between positions, since this quantity reflects the variations in chloride distribution. It is evident that the observed differences in the chloride content at different positions, as indicated by the results in Table I, are statistically significant at the first two samplings. Since the mean square between positions decreases between successive samplings, it is evident that the chloride becomes more uniformly distributed during the maturation period. In the group of sides sampled after smoking, the difference between positions is not significant, indicating the absence of a systematic gradient in chloride concentration.

The interaction position × plants was also significant at both samplings for the entire lot of 44 sides. This indicates that there was not a uniform gradient in chloride concentration from one position to another in the product of all plants. It is possible that this could be attributed to the different pumping practices, etc., followed in different plants. Since the sides from the different plants varied in age from two to 11 days from cure at the time

of the first sampling, it may be that the different gradients merely reflect the effect of ageing.

Chloride Distribution in Relation to Curing Practice

Before attempting to establish a quantitative relation between the chloride distribution and the age of the sides, some consideration had to be given to the effect of variations in the curing practices, in order that these factors could be taken into account. The standard deviation between the positions for both sides received from each plant was used as an estimate of chloride distribution. Although the standard deviations between all three positions were used initially, it was found, as the computations proceeded, that the difference between Positions 1 and 3 was an equally sensitive measure of the variations in chloride content. The standard deviation or difference between the positions observed for all sides at the first sampling was correlated with the known curing factors, as outlined in the first part of Table III. The results of the second sampling were not included, since the influence of age tends to minimize any difference attributable to the various curing practices.

Examination of these correlation coefficients shows that only the coefficient relating the standard deviation between positions and the number of stitches used per side attains the 5% level of significance. In fact, the other coefficients relating quantities involving the number of stitches per side, although not significant, were much larger than the correlations between the standard deviation and other quantities, and probably would have been significant had the sides been analysed immediately after cure. It is therefore concluded that chloride distribution is influenced more by the number of stitches used in pumping than by the observed variations in the chloride content of either of the pickles or by the number of days in cure.

In an earlier paper (6), it was shown that the number of stitches injected per side was the only known curing practice that was correlated with the over-all chloride content of bacon. Since the chloride content at Position 1 should be affected primarily by the tank curing practice, and that at Positions 2 and 3 by the pumping practice, correlation coefficients were computed between the chloride contents at these positions, for the first sampling, and the known properties of the pumping and tank curing practices. The results obtained are shown in the second part of Table III. Although none of the correlation coefficients are significant, their magnitude suggests that the number of stitches per side is the most influential factor of those studied in determining the chloride content at Position 2. It appears, therefore, that within the limits used in practice, similar amounts of chloride are taken up from the tank pickle regardless of its concentration or period in cure. In these circumstances, pumping the sides would be expected not only to reduce the variation in chloride content between the outside and the centre positions, but also to increase the total amount in the bacon. Nevertheless, correlation coefficients failed to demonstrate a definite relation between chloride content and chloride distribution.

TABLE III

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE DISTRIBUTION AND CERTAIN CURING PRACTICES

Quantities correlated	D.F.	r
Sodium chloride distribution in relation to curing practice		
Standard deviation of sodium chloride content at all 3 positions with:		
No. of stitches per side	20	−0.42*
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
Difference in sodium chloride content between Positions 1 and 3 with:		
No. of stitches per side	20	−0.42*
Days in cure	20	−0.14
Sodium chloride in tank pickle	20	+0.01
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
No. of stitches per side, independent of days in cure	19	−0.41
No. of days in cure, independent of number of stitches per side	19	−0.08
Sodium chloride content in relation to curing practice		
Sodium chloride content of meat at Position No. 1 with:		
Days in cure	19	+0.14
Sodium chloride in tank pickle	19	+0.01
Sodium chloride in tank pickle × days in cure	19	+0.14
Sodium chloride content of meat at Position No. 2 with:		
No. of stitches per side	20	+0.37
No. of stitches per side × sodium chloride in pump pickle	19	+0.34
Sodium chloride content of meat at Position No. 3 with:		
No. of stitches per side × sodium chloride in pump pickle	19	+0.07
Relation between distribution and content of sodium chloride		
Standard deviation in sodium chloride content at all 3 positions with mean chloride content of all 3 positions for:		
First sampling by plants	20	−0.18
First sampling by sides	42	−0.11
Second sampling by plants	20	−0.36
Second sampling by sides	42	−0.29

* *Indicates 5% level of significance.*

Comparison of Pumped and Unpumped Sides

The results presented in this and earlier papers (5, 6) have indicated the importance of the method of pumping, in determining the concentration of curing salts in bacon. To confirm this a few preliminary experiments were made on the composition of pumped and unpumped sides cured simultaneously in the same tank in a packing plant. Four pumped and two unpumped sides were sampled and analysed, by the methods previously described (11), immediately on removal from cure and again 17 days later. The mean com-

position of each group of sides, with respect to sodium chloride, nitrate, and nitrite, appears in the first part of Table IV, and the chloride distribution, as shown by the sodium chloride content at the different positions, in the second part. Statistical methods were not applied to these few results, but since the different sides in each group varied considerably, only relatively large differences can be considered significant.

TABLE IV

COMPOSITION OF, AND CHLORIDE DISTRIBUTION IN, PUMPED AND UNPUMPED SIDES

Constituent	Pumped		Unpumped	
	1st sampling	2nd sampling	1st sampling	2nd sampling
Constituent—				
Sodium chloride, %	2.61	3.68	0.56	1.93
Sodium nitrate, %	0.16	0.16	0.008	0.028
Sodium nitrite, p.p.m.	17	34	0.8	27
Sodium chloride distribution				
Position—				
1	4.92	2.96	3.15	2.28
2	3.08	2.91	0.82	1.26
3	3.41	3.48	0.46	1.05

It is evident that the slice of ham analysed at the first sampling contained less of all three salts than at the second sampling. In general, this apparent increase in the content of the curing salts must be attributed to differences between positions rather than a change with time (6). Nevertheless, the proportionately greater increase in the nitrite content of the unpumped sides between samplings suggested a real change during storage. This confirms an earlier finding (6) that nitrite is produced in sides having a low nitrite content initially.

Comparison of the values obtained at the second sampling, when a reasonably uniform distribution of the salts has occurred, indicates that pumping contributes about half the chloride and 80% of the nitrate found in the bacon. The chloride content at the three positions for the second samplings suggests a relatively uniform distribution throughout the hams of the pumped sides, while the unpumped sides still had about twice as much chloride at the position adjacent to the tank pickle, as at the central positions. The importance of pumping in determining both the concentration of the salts and their distribution in the sides is apparent.

Effect of Pumping and Ageing

It was concluded from the results already presented that the number of stitches used for pumping and the age from cure were the principal factors affecting the distribution of salts in hams of Wiltshire sides. In order to obtain

a quantitative estimate of the effect of these two factors, a partial regression equation was computed for the standard deviation between positions on number of stitches used in pumping and the number of days from cure over all samplings. This equation was found to be:

$$Y = 4.64 - 0.074\,S - 0.126\,N$$

where

Y = standard deviation between salt content at different positions,

S = number of stitches used per side

and

N = number of days from cure.

Although this equation shows the extent to which the variations in chloride content between different positions decrease with increase in number of stitches per side and days from cure, it is also important to determine the amount of the total variance accounted for in this way. The mean square for the observed variations in Y (standard deviation between positions) was found to be 1.42, and after subtracting the variance accounted for by the above equation, the residual mean square was 0.70. This shows that variations in pumping practice and age of the sides accounted for about half of the observed variance.

From a practical standpoint, there is no apparent advantage in reducing the variations in salt content within an individual side to less than those between different sides produced under the same conditions in the same plant. Reference to Table II shows that the mean square between sides over all samples at the second sampling was 0.80.

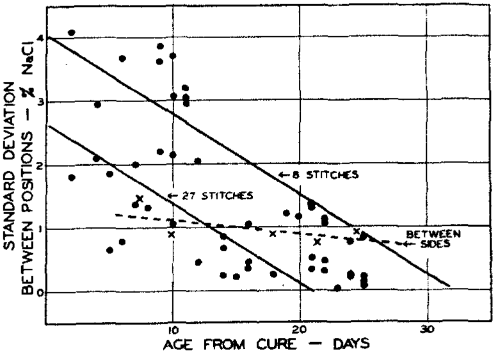

FIG. 2. *Effect of number of stitches used in pumping, and the age of the side on the distribution of chloride.*

Using this criterion, it is possible to predict the time required, following various pumping practices, to obtain a satisfactory chloride distribution throughout the ham of a Wiltshire side. In order to do this the equation given earlier was used to construct Fig. 2, in which the estimated standard deviation between positions is plotted against the age of the sides from cure. The upper line represents the results with the minimum (eight), and the lower, with the maximum (i.e., 27) number of stitches used for pumping the sides studied in this investigation. The experimental points, representing the mean value for the two sides received from each plant, are plotted for comparison. The crosses and broken line indicate the level of the standard deviation between sides, computed from the mean square given in Table II, for sides of various ages from cure. The time required for the standard deviation between positions to fall to this level represents that required to obtain a satisfactory chloride distribution. The figure suggests that this level could be attained in about 12 and 26 days, respectively, for sides receiving the maximum and minimum number of stitches ordinarily used in pumping. Since Canadian sides never arrive in England in less than 12, and on the average 18, days after removal from cure, it appears that they must be satisfactory from the standpoint of chloride distribution before they reach the consumer.

Discussion

Considerable work has been done on the rate of salt penetration into meat, and its subsequent distribution within the cured product. An extensive review of the literature on this subject is unnecessary, but it is of interest to consider the findings of certain investigators in relation to those obtained in this study. Reduced to essentials, the present results show that the number of stitches used in pumping and the age of the sides from cure were the only factors of those studied found to affect the chloride distribution in Wiltshire bacon as manufactured by current methods.

Banfield and Callow (2) have shown that the decrease in electrical resistance during cure was less marked in unpumped than in pumped sides. This implies that the unpumped sides contained less salt, a result confirmed by analysis after maturation, although the small difference observed for tank cured sides is doubtless of little practical consequence. These investigators used the backs, and since the lean portion at this point is smaller than that of the ham, a more uniform penetration and distribution of the chloride is to be expected, and the effect of pumping consequently less marked.

Besley and Hiner (3) have shown that the various muscles in unpumped lamb legs absorb salt differentially, and claim that even larger differences occur between the different muscles in hams. The results of the present study suggest that where a reasonably satisfactory pumping practice is used, with respect to both the number and position of the injections, the differential absorption of chloride by different muscles would have little effect on the chloride distribution. They also found that the number of days in cure was the principal factor affecting the chloride content and distribution between different

muscles. Since these lamb legs were both unpumped and cured for a period far in excess of that generally used for curing Wiltshire bacon, these findings do not appear to contradict those obtained in these investigations (3).

Miller and Ziegler (8) studied the chloride distribution in hams, using sampling and statistical methods comparable to those used in this study. The hams, however, were not pumped and the length of the curing period was considerably longer than that used for Wiltshire sides. Their results indicate that following a brine-curing period of 30 days, a holding period of another 30 days at 37° F. was required to reduce the coefficient of variability between positions to about 20% or less. This variability corresponds to a standard deviation of about 0.80% when interpreted in terms of the mean sodium chloride content, expressed on a "wet" basis, as observed in these studies and reported in Table I and previously (6). Using the equation given earlier, with $S = 0$, since no pickle was injected, and $Y = 0.8$, a value of about 30 is obtained for N. It is indeed surprising that the results of these two independent investigations agree so closely, considering that they represent work with pumped sides and unpumped hams, cured for widely different periods, and subsequently stored at somewhat different temperatures.

Acknowledgments

The authors wish again to express thanks to the firms and individuals mentioned in the first paper of this series, and particularly to Messrs. A. E. Chadderton and G. N. Seed, laboratory assistants, who were responsible for most of the sampling, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on the statistical treatment of the results.

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CANADIAN WILTSHIRE BACON

XI. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT[1]

By W. H. White[2], W. H. Cook[2], and C. A. Winkler[3]

Abstract

Small cuts of matured Wiltshire bacon were held at temperatures of 20, 40, 50, 60, 70, and 80° C. for periods of 5, 10, 20, and 40 hours. When the nitrite content after treatment was plotted against the temperature, the results showed that the highest nitrite contents were obtained at 40° C., the nitrite content decreased logarithmically between 40 and 80° C., and the slope of these curves increased with increasing periods of treatment. The curves representing the different periods of treatment intersect in the region of 55° C., where the nitrite content was approximately normal. It appears, therefore, that an increase in nitrite content above the normal level occurred at temperatures below 55° C. and a decrease at higher temperatures. This decrease in nitrite nitrogen above 55° C. may be attributed to direct loss, oxidation, or reaction with the constituents of the meat. The increase in nitrite observed at temperatures below 55° C. suggests that time and temperature were not alone responsible for the observed changes, since comparable increases in nitrite were not observed in commercial cuts. It may be that the proportionately greater surface exposed to the air in these small samples may have had some effect, such as an enhanced aerobic bacterial action.

Introduction

The characteristic colour of bacon is attributed primarily to the combination of nitrite with the muscle haemoglobin to form nitrosohaemoglobin. This compound is believed to be converted to a more stable form, termed nitrosohaemochromogen, when the meat is cooked, and this change may also occur to some extent during smoking at higher temperatures, i.e., above about 55° C. In earlier papers (7, 8) of this series, it has been shown that the pH and nitrite content of the bacon affected colour and colour stability. It may be that these influential factors act either by determining the rate or extent of the reaction producing nitrosohaemoglobin, or by an independent effect on the colour of the bacon. The nitrite content of bacon may not reflect its nitrosohaemoglobin content, but as a factor affecting colour it deserves consideration. It has also been shown (3, 6) that the nitrite content of certain sides, through bacterial action or otherwise, may increase during holding after cure, and decrease slightly during smoking at about 45° C. (3).

An investigation was, therefore, undertaken to determine the effect of heating Wiltshire bacon, for various periods at different temperatures, on its nitrite content and colour. This paper deals with the changes in nitrite content.

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Material, Design, and Method

Since the earlier studies (3) showed that different sides, treated in the same manner, differed significantly in nitrite content, it seemed desirable to have different sides cured in several different plants represented in the experiment. The available material consisted of "backs", cut from two sides (from different pigs) cured in each of eight different packing plants. These sides represented bacon of various ages, having been removed from cure and held at temperatures of 1 to 3° C. for periods of from 23 to 25 days before being sampled for these studies.

The experiment was arranged in a factorial design (4), involving: 4 times of treatment, namely, 5, 10, 20, and 40 hr.; 6 temperatures, namely 20, 40, 50, 60, 70, and 80° C.; and 4 replications of each treatment. The 96 pieces required were obtained as follows: The 16 backs were randomized over the 4 replicates and 4 times of treatment; each back was then divided into 6 approximately equal parts of about 100 to 200 gm. each, and these pieces were randomized among the 6 temperatures within times. It is obvious that this design yields more precise information on the effect of temperature than of time, since the significance of the observed differences with temperature is tested by comparison with the analytical and sampling error within sides, while the differences between times of treatment must be tested by comparison with the difference between sides. Within the limitations of available material, time, and facilities, it seemed more desirable to obtain precise information on the effect of temperature.

The samples were first trimmed free of the cut portions of the ribs and the layer of back fat. The individual pieces were weighed, wrapped in lightly waxed glassine, and then in kraft paper. The individual packages to be heated at a given temperature were wrapped into a single package. These operations were performed in a laboratory at about 25° C. The samples were placed in ovens at the required temperatures, removed after the required period of time, and weighed. The surface layers were trimmed off, suitable samples taken for colour measurements, and the remainder, trimmed free of fat and connective tissue, was ground and mixed by several passages through a food chopper, bottled, and frozen at −29° C. The samples were thawed as required, analysed by the method previously described (5), and the results treated by statistical methods (4).

Results

The loss of weight during heating was a function of the time and temperature of treatment. At the higher temperatures these losses represented not only a loss of moisture but also of a certain amount of fat that melted and was absorbed by, or lost from, the paper wrapping. These figures were used for computing the amount of nitrite in terms of the original sample weight, but otherwise are of little significance in the present study.

The mean nitrite content of the four samples treated under each condition is reported in Table I. In considering these results, it must be remembered that there was considerable variation between the individual pieces from different sides treated in the same way. This, together with the fact that the nitrite content after the different treatments also varied widely, made it necessary to employ statistical methods to test the significance of the observed differences.

TABLE I

MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS

(As p.p.m. in initial weight)

Period of heat treatment, hr.	Temperature of heat treatment, °C.					
	20*	40	50	60	70	80
5	25.81	22.16	19.73	16.94	20.16	14.38
10	29.21	41.88	41.40	15.02	8.62	5.49
20	179.52	191.25	51.35	10.20	5.76	2.36
40	—	404.75	33.95	9.41	3.82	1.32

* *Samples treated at this temperature considered separately in subsequent statistical analysis.*

The values reported in Table I show that the samples varied in nitrite content from about one to over 400 p.p.m. The deviations of the individual values from the reported means were approximately proportional to the mean. Since results exhibiting a variability of this magnitude cannot be used directly for an analysis of variance (2), they were first converted into logarithms of the nitrite content in parts per million before the statistical computations were undertaken. All numerical values subsequently reported are on this basis.

The samples held at 20° C. were used to determine the changes in nitrite content that occur at room temperature. Unfortunately, the samples that were to be held for 40 hr. at this temperature were removed and ground after 20 hr., so that 8 samples were treated for 20 hr. at this temperature but none for 40 hr. This loss was not serious, however, since it seemed desirable, in making the statistical analyses, to treat the results obtained at 20° C. separately from those obtained at higher temperatures.

The results of an analysis of variance are reported in Table II. It is evident that the difference between the nitrite content of the samples held at 20° C. is highly significant. This shows that the increase in nitrite content, as shown in Table I, exceeds the variations observed between different sides, even within a 20-hr. period. The remaining samples, treated at higher temperatures, were considered together, and for these the average change in nitrite content with time is not significantly greater than the differences between the different sides that were treated similarly, but is significantly greater than the sampling and analytical error within sides. From

TABLE II

ANALYSIS OF VARIANCE. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT OF BACON

(Values in terms of logarithms of nitrite content in p.p.m.)

Variance attributable to	Degrees of freedom	Mean square
Samples heated at 20° C.		
Between times of treatment	2	1.426**
Between sides within times	13	0.124
Samples heated at 40 to 80° C.		
Between times of treatment	3	0.491
Between sides within times	12	0.509
Between treatment temperatures	4	4.812**
Differential effect of temperature × time	12	0.597**
Sampling and analytical error within sides	48	0.035

** *Indicates 1% level of significance.*

this it is concluded that variations in the properties of the individual piece or side, or in their bacterial content or flora, determine, to a large extent, the concentration of nitrite present after exposure to heat treatments comparable to that of smoking. This conclusion is in general agreement with results reported previously (3, 6).

The differences between the nitrite content following a given period of treatment at different temperatures of treatment, can be determined more exactly, since the same sides were represented at all temperatures. It is evident from Table II that the differences between temperatures and the variance attributable to the interaction, temperature × time, were both highly significant. This means that not only is the nitrite content a function of the temperature of treatment, but also that temperature has a differential effect on the nitrite content, depending upon the duration of treatment.

The direction of these significant changes in nitrite content under different conditions is evident from the results reported in Table I, but is shown more clearly in graphical form in Fig. 1. In this figure the nitrite content is plotted on a logarithmic scale against the temperature of the treatment. The four curves shown represent the different periods of treatment. Since the results obtained at 20° C. were considered separately in the statistical analyses, and since nothing is known of the changes that occur in the temperature range from 20° to 40° C., the points obtained at these two temperatures have been joined by a broken line, although the nitrite-temperature relation is doubtless curved in this range.

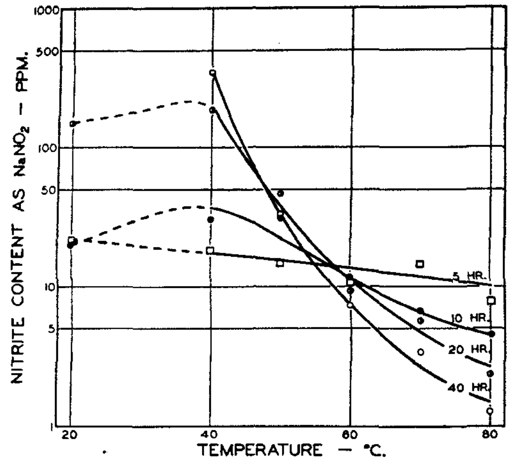

FIG. 1. *Effect of time and temperature of heating on the nitrite content of Wiltshire bacon.*

It would appear that the nitrite content, following a given period of treatment, is generally higher at 40° C. than at 20° C. Above 40° C. the nitrite content falls off more or less logarithmically as the temperature increases. Extending the period of treatment increases the nitrite content at 40° C., and decreases it at 80° C. The curves representing the various periods of heating, therefore, differ significantly in slope (Table II), and intersect one another in the region of 55° C., where the nitrite contents were approximately normal.

Discussion

Callow (1) has found that the nitrite content of small cuts of bacon may increase during storage for several weeks after curing. He found that the maximum nitrite concentration occurred at 5° C. and attributed the increase to bacterial action. The experiments reported in this paper were made over shorter periods at higher temperatures. In the present investigation the highest nitrite concentration occurred at 40° C., although the results at 20° C. suggest the possibility of a maximum nitrite production between 20 and 40° C. Since the optimum temperature for most bacteria lies between 20 and 40° C., it would seem reasonable to expect, if nitrite production is dependent on microbial activity, the maximum production of nitrite in this temperature range, rather than at cellar temperature.

In the main series of experiments (3), involving whole sides or large cuts, comparable increases in nitrite content were never observed, even in an individual side, during 10 days' storage at 1.1° C., smoking overnight at 40 to 45° C., and holding at room temperature for periods of 12 to 24 hr. In fact, smoking under these conditions tended to reduce rather than increase the nitrite content. These results suggest that time and temperature of treatment were not alone responsible for the increase in nitrite nitrogen observed in the present study.

It is possible that the proportionately greater surface exposed to the air in these small cuts may have been responsible for the observed behaviour. Certain direct evidence favouring this hypothesis was obtained from the observation that ground bacon stored at 4° C. showed a large increase in nitrite content within 36 hr., while the same bacon in the whole side showed no significant change. It appears, therefore, that the present results represent the effect of time and temperature under conditions that probably exaggerate their influence on nitrite production, as compared with those existing in commercial practice.

Although the results indicate that the nitrite content increased at temperatures below about 55° C., the agency responsible for these changes cannot be determined from these experiments. The accelerating effect of exposure to the atmosphere suggests that aerobic bacteria are responsible for nitrite production, but it is also possible that certain constituents or enzymes of the meat are capable of reducing the nitrate present to nitrite. Further work has been undertaken to determine the factors responsible for the observed increase in nitrite concentration.

Since protein coagulation occurs at temperatures in the vicinity of 55° C., bacterial and enzymic activity would be reduced to negligible proportions by exposure to higher temperatures. At temperatures above 55° C., the observed decrease in nitrite content may be attributed to chemical reactions causing a direct loss, oxidation, or combination of nitrite with the constituents of the meat.

Acknowledgments

The authors are indebted to the firms and individuals mentioned in the first paper of this series, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on the statistical treatment of the results.

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FURTHER EXPERIMENTS ON THE USE OF PHENOTHIAZINE AS AN ANTHELMINTIC FOR SHEEP[1]

By W. E. Swales[2]

Abstract

The development and production of a disintegrating tablet as a practical means of employing phenothiazine as an anthelmintic for sheep are further described. Continued critical tests have shown high efficiencies against *Oesophagostomum*, *Haemonchus*, *Ostertagia*, *Monodontus*, *Nematodirus*, *Cooperia*, *Trichostrongylus*, and *Chabertia*. Tests against single infections with *Ostertagia* continued to show the complete efficiency of this form of treatment against this parasite. The periods of excretion of destroyed parasites with the faeces are noted. Single doses of phenothiazine did not anaematize animals under treatment.

The value of commercial phenothiazine as an anthelmintic for sheep was demonstrated during 1939. Harwood *et al.* (5) showed that, when mixed with food, the chemical was eaten by fasted sheep and resulted in the removal of a high percentage of hookworms (*Monodontus trigonocephalus*), nodular worms (*Oesophagostomum columbianum*) and probably stomach worms (*Haemonchus contortus* and *Ostertagia circumcincta*). Swales (8) reported tests showing high efficiency against the above mentioned species, and also demonstrating the efficient removal of *Trichostrongylus* spp., *Cooperia* spp., *Nematodirus* spp., and *Chabertia ovina*. During these tests a practical method was evolved for administering the drug to sheep; chemicals were ground and mixed with commercial phenothiazine and the mixture then compressed into hard tablets or slugs. Upon being placed in water or in the rumen of a sheep the tablet rapidly disintegrated and small particles of the insoluble phenothiazine were immediately dispersed. This method did not require prior fasting of the animals, required only about one-half minute to administer a therapeutic dose, and appeared to enhance the anthelmintic effect.

Roberts (6) and Gordon and Whitten (2, 3) reported tests of phenothiazine which demonstrated high efficiency against *H. contortus* and variable efficiency against *O. columbianum* and *Trichostrongylus* spp. Roberts administered relatively small doses of the chemical as a suspension in liquid paraffin, relying upon the prior stimulation of the reflex closure of the oesophageal groove by a solution of copper sulphate to direct the dose into the abomasum. In these experiments the efficiency against *O. columbianum*, with 4 gm. of phenothiazine in each dose, ranged from 8.8 to 94.7% in seven lambs. Roberts (7) also made extensive tests using a commercial preparation of phenothiazine ("Thiox"). He concluded that at a dose rate of 0.15 gm. per pound body

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[2] *Associate Animal Pathologist, Science Service, Dominion Department of Agriculture.*

weight this drug gave fairly satisfactory results against *O. columbianum*, and at a dose rate of 0.4 gm. the results were very good in grown sheep but not equally good in lambs. He found the bulk of the larger dose to be an objectionable feature. During these tests Roberts noted a high efficiency against *H. contortus* but no effect against *Trichuris* spp.

Habermann and Harwood (4) later reported further tests using recrystallized phenothiazine. In doses from 15.6 to 20.0 gm., an average efficacy of 21.4% for *Monodontus (Bunostomum)*, 90.1% for *Oesophagostomum*, 47.9% for *Chabertia*, 100% for *Haemonchus* and 20% for *Ostertagia* was demonstrated. In doses of 22.0 to 31.75 gm. the efficacy was 95.1% for *Monodontus*, 83.9% for *Oesophagostomum*, 93.8% for *Chabertia*, 80% for *Haemonchus* and 40% for *Ostertagia*. (No *Ostertagia* were recovered from faeces prior to necropsy, the estimation being made from prior examination of ova). Doses of 20 gm. were only slightly effective in removing *Cooperia* and partially effective in removing *Trichostrongylus*; however, a dose of 25 gm. removed 85.2% of 338 *Trichostrongylus* from one animal.

The work previously reported (8) was continued and extended in an effort to determine the practicability of the use of this anthelmintic in commercial sheep flocks.

Commercial Phenothiazine in the Form of Compressed Tablets

Following the preliminary description of the compressed tablet, attempts were made to improve this method of medication. However, tests have shown that only minor changes were necessary in the formula, which is now as follows:—

Commercial phenothiazine	—	80 parts
Starch	—	8 parts
Sodium bicarbonate	—	5 parts
Tartaric acid	—	4 parts
Sodium choleate	—	2 parts
Phenolphthalein (U.S.P.)	—	1 part

In preparing tablets for experimental use a satisfactory routine was developed. The ingredients are weighed into a one-gallon ball or pebble mill so that one load contains 800 gm. of the formula. Four pounds of pebbles and a heavy chain are used for grinding and mixing in the mill. The mill is turned for 15 min. at 70 r.p.m. The powder mixture is separated from the pebbles through a screen and is compressed into lumps by means of a plant-juice extracting press at 2,000 lb./sq. in. The lumps are granulated through a 10 mesh/inch screen. The granular material is compressed into the final tablet by means of a foot-operated press which produces a leverage on a piston of 11.5 : 1 by means of a heavy curved pedal lever. The material is measured into a die, which is a heavy walled steel cylinder, 3 in. long by $\frac{3}{4}$ in. inside diameter at the top increasing uniformly to a diameter of 51/64 in. at the bottom; the compression is obtained by a preliminary pressure on the lever

followed by a forceful kick. The tablet is then expressed by moving the base plate and thus allowing the piston to push the tablet through an aperture in the base plate and into a receptacle. The tablets made in this way are almost cylindrical (2.3% attenuation), have a hard, polished surface and are convex at each end. A single operator can make up to 200 tablets per hour. The above mentioned machine will produce tablets from 9 to 12.5 gm. in weight, depending on the measure used for the granulated material. A tablet weighing 12.5 gm. is approximately 1.5 in. in length.

The administration of the tablets presents no difficulties. A simple metal speculum, with bars from 2¼ to 2½ in. apart, is used for adult animals; the tablets are placed, one at a time, over the back of the animal's tongue, the speculum being immediately withdrawn after each tablet is administered.

The administration of four tablets to an adult sheep usually occupies about half a minute if the animal is held correctly by an assistant.

Further Tests of Phenothiazine (Tablets)

Further tests were conducted in a similar manner to those previously reported (8). None of the animals were fasted prior to treatment and they had access to food and water immediately afterwards. About four hours after administration of the dose a specially constructed harness was fitted on the animal and a canvas bag attached over the hind-quarters so that all faeces were collected.

For three days previous to treatment and for four days afterwards, haemoglobin estimations (Sahli) and total erythrocyte counts were made twice daily on Animals 1 to 5 inclusive. This was done in order to check the possibility that single large doses of phenothiazine could produce a transitory anaemia, as reported by Thomas *et al.* in rabbits (9) and by DeEds *et al.* in man (1). In none of these animals was there any indication of a decrease in haemoglobin or in the number of erythrocytes. None of the animals showed any symptom of toxic effects of the dose, and no lesions attributable to the treatment were discovered at autopsy.

The results of the tests on nine animals are presented in Table I, and the post-treatment periods in which the removed parasites were found in the faeces are shown in Table II.

It is of interest to note that of the 85 residual *Monodontus*, only 26 were females, all but two of this sex being found in Animal No. 9. Most of the specimens of *Monodontus* removed during the first 24 hr. were females, the reverse being true for the subsequent periods. This supports the similar observation made during the previous tests and may be of some significance in the determination of the mode of anthelmintic action of phenothiazine.

Tests against single infections of *Ostertagia circumcincta* have been made. Four lambs, previously used for other work, and which were free of other strongyloid nematode parasites, enabled tests to be made without the necessity of sacrificing the subject after each treatment. The infections in Animals B,

TABLE I

ANTHELMINTIC EFFECT OF PHENOTHIAZINE ON INFECTED SHEEP

Animal	Weight (lb.)	Parasites removed by *treatment* and recovered from faeces												Parasites recovered at necropsy												
		Strongyloid ova (per gram)	Dose (gm. tablet formula)	Date	*Haemonchus*	*Ostertagia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Chabertia*	*Oesophagostomum*	Date of necropsy	*Haemonchus*	*Ostertagia*	*Moniezia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Strongyloides*	*Trichuris*	*Chabertia*	*Oesophagostomum*	*Capillaria*
1 Aged F.	125	1,500	62	11/9/39	0	0	46	0	0	0	0	9	22/9/39	0	0	0	0	0	0	0	0	0	0	0	10
2 Aged F.	114	–	50	25/9/39	0	0	12	0	0	0	0	2	3/10/39	0	0	0	0	0	0	0	0	0	0	0	0
3 Lamb M.	50	4,500	25	5/9/39	110	12	4	950	20	1,045	0	14	12/10/39	0	0	7	4	122	0	311	30	0	0	1	0
4 Lamb M.	54	1,800	27	15/10/39	31	130	71	27	356	664	10	15	19/10/39	0	0	3	2	103	0	107	60	3	0	0	0
5 Lamb F.	49	1,330	25	19/10/39	11	22	105	12	870	450	9	11	25/10/39	0	0	1	30	0	0	1,080	80	1	0	1	0
6 Aged F.	96	–	48	19/11/39	0	0	23	1	40	70	0	1	23/11/39	0	0	1	0	0	230	0	20	0	0	0	1
7 Lamb M.	64	1,200	32	18/12/39	0	124	260	4	0	110	16	25	12/1/40	0	0	0	1	29	0	110	10	0	1	2	0
8 Lamb F.	66	200	33	8/1/40	7	12	25	163	0	143	6	0	12/1/40	0	0	2	8	200	0	67	0	23	0	0	0
9 Lamb M.	62	1,200	31	29/1/40	0	10	167	0	21	650	10	28	7/2/40	0	0	0	40	0	0	70	0	0	2	0	0
Total worms					159	310	713	1,157	1,307	7,332	51	105		0	0	14	85	454	230	1,745	200	27	3	4	
Estimated average percentage efficiency of treatment														100	100	0	89	72	85	81	0	0	94	96	

TABLE II

THE POST-TREATMENT RECOVERY OF PARASITES FROM THE FAECES

	Percentages of total for each species			
	8 – 24 hr.	24 – 36 hr.	36 – 48 hr.	48 – 72 hr.
Haemonchus	97.5	1.9	0.6	0
Ostertagia	66.1	33.9	0	0
Monodontus	64.1	16.3	12.5	7.2
Nematodirus	60.8	5.8	33.1	0.3
Cooperia	96.9	3.1	0	0
Trichostrongylus	53.2	38.1	8.1	0.6
Chabertia	43.1	27.5	29.4	0
Oesophagostomum	72.4	16.2	11.4	0

C, and D were induced by doses of 5,000, 50,000, and 50,000 infective larvae of *O. circumcincta* respectively, the origin of the larvae being faeces from Animal A which acquired the infection on a test plot. Before artificial infection the cultures were carefully examined for the presence of larvae of other species, with negative results. The results of the treatments are shown in Table III.

TABLE III

EFFECT OF PHENOTHIAZINE ON LAMBS INFECTED WITH *Ostertagia circumcincta*

Animal	Weight, lb.	Dose (gm. tablet formula)	Date	Infection (ova/gm.)	Post-treatment tests*
A	90½	36	6/11/39	250–900	Tests negative 8/11/39 to 29/12/39
B	96	48	13/12/39	200–400	Tests negative 14/12/39 to 19/2/40
C	61	30	3/1/40	200	Tests negative 4/1/40 to 1/3/40†
D	100	50	3/1/40	200–600	Tests negative 4/1/40 to 4/3/40

* *All post-treatment tests were concentration and sugar flotation methods using 15 gm. of faeces.*

† *Of 13 tests, two revealed the presence of 2 and 3 strongyloid ova respectively (25/1/40 and 1/2/40).*

The post-treatment faeces from A and C were not examined for the presence of worms. Sixty-five specimens of *Ostertagia* were recovered from Animal B, 20 in the first 19 hours, 44 between the 19th and 24th hours, and one between the 24th and 27th hours. The faeces from Animal D contained 119 specimens, of which 69 were recovered in the first 19 hours and 50 between the 19th and 24th hours. It is probable that these worms represented only part of the total, as many may have become unrecognizable before being excreted with the faeces.

Discussion of Results

The commercial phenothiazine used in the experiments included in Table I was approximately 95% pure phenothiazine. Thus the doses used, based on the pure form, ranged from 19.0 to 46.5 gm. and averaged approximately

28.0 gm. of the pure chemical. The consistently good results indicate that the tablet form of the dose, which rapidly disintegrates into very small particles in the stomachs, enhances the anthelmintic efficiency of the phenothiazine. The definite laxative effect of the dose is apparent within a few hours, and particles of phenothiazine can be detected in the faeces between the eighth and twelfth hours after dosing. Although, in these experiments, only worms actually recovered from the faeces are considered in comparison with those remaining in the intestinal tract in estimating results, the apparent efficacies against *Oesophagostomum*, *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Cooperia* are significantly greater than those obtained by Habermann and Harwood (4) with recrystallized phenothiazine given in feed or in capsules.

The complete removal of *Ostertagia* by the described method supports previous findings. It is probable that a considerably smaller dose would be adequate for this purpose. The application of the treatment of ostertagiasis will be of greater importance in other countries including Great Britain, as this parasite has not, so far, been found to be of economic importance in Canada.

Acknowledgments

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THE FATE OF PHENOTHIAZINE IN THE SHEEP[1]

BY H. B. COLLIER[2]

Abstract

Methods have been devised for the detection and estimation of phenothiazine and its derivatives in the faeces, urine, blood, and milk of sheep, following oral administration of the drug.

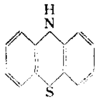
Only unchanged phenothiazine was detected in the faeces. In the urine was found a conjugate which was hydrolysed by strong acid in the cold. The conjugate was tentatively identified as potassium leuco phenothiazone sulphate; it was also present in the blood serum and the milk.

Catalase is inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazone.

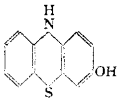
Introduction

The fate of orally administered phenothiazine in the rat, rabbit, and human has been discussed by DeEds *et al.* (2). They found that the substance was excreted in the urine as thionol, leuco thionol, and unidentified conjugates of phenothiazine and of leuco thionol.

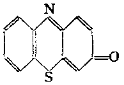
Phenothiazine is highly insoluble in water, but is soluble in alcohol and the fat solvents, crystallizing out in flat, pale yellow leaflets. It is changed to phenothiazone under mild oxidizing conditions and to thionol by stronger oxidizing agents. Both these derivatives are slightly soluble in water, giving an intense magenta colour, and both may be reduced to colourless leuco compounds. Eddy and DeEds (3) have described a quantitative method for phenothiazine, based on oxidation by bromine, and colorimetric estimation of the red colour produced.



PHENOTHIAZINE
$C_{12}H_9NS$



LEUCO PHENOTHIAZONE
$C_{12}H_9NSO$



PHENOTHIAZONE
$C_{12}H_7NSO$

The present paper describes the examination of the faeces, urine, peripheral blood, and milk of sheep for phenothiazine and its derivatives, subsequent to administration of phenothiazine in tablet form, as described by Swales (7, 8). It seems highly desirable to identify the derivatives formed from phenothiazine in each species examined, in the hope that the findings may throw light on the pharmacology of the drug.

Following the identification of the chief excretory products in the sheep, quantitative methods have been devised and used to measure the rate of absorption and excretion. These methods are believed to be adequate for this purpose, although further work will doubtless result in refinements of the technique.

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[2] *Research Associate.*

Qualitative Examination

Faeces

Faeces from treated animals were extracted with hot water, but the filtered extract failed to show any colour, even after treatment with hydrogen peroxide or strong hydrochloric acid. This indicated that the water soluble oxidation products of phenothiazine, or their conjugates, such as may be detected in the urine by this procedure, were absent from the faeces.

Extraction with hot alcohol, followed by treatment with bromine water, yielded an intense red colour, suggesting the presence of unchanged phenothiazine. (Normal faeces gave no colour when treated similarly.) In fact, particles of phenothiazine were visible upon microscopic examination of the faeces.

Urine

The examination of the urine yielded results essentially similar to those described by DeEds *et al.* The urine when voided was of normal colour, but quickly turned red on exposure to the air. (If the coloured urine was allowed to stand in a flask, all but the surface layer slowly bleached to the normal colour, indicating reduction of the red compound.) The red pigment was extractable by chloroform and was assumed to be either phenothiazone or thionol.

Acidification of the urine with strong hydrochloric acid resulted in a very intense purple colour and the formation of a precipitate; this was apparently due to hydrolysis of a conjugate. The precipitate was soluble in alcohol and was oxidized by bromine to a red compound; it was therefore believed to be phenothiazine, as found by DeEds *et al.* However, the analysis and properties of the precipitate indicated, as described below, that it was not phenothiazine but a derivative.

Cooling of the urine caused the formation of a bulky, light coloured precipitate. This was found to be very soluble in hot water, and crystallized as sheaves of colourless needles. The crystals were soluble to the extent of about 1% in water at 20° C., and insoluble in alcohol and fat solvents. Treatment of the aqueous solution with strong hydrochloric acid and hydrogen peroxide gave a precipitate and an intense purple colour. The compound was therefore believed to be a conjugate; it was hydrolysed by strong acids, but not by weak acids or by alkalies.

A quantity of the conjugate was isolated from urine and recrystallized four times from hot water. The crystals did not melt sharply but darkened at about 220° C. A sample was submitted for analysis to Prof. Leslie Young of the Department of Biochemistry, University of Toronto. He reported as follows:

> C = 43.12, 43.27%. H = 2.71, 2.74%. Ash = 23.85%. Potassium and sulphate were detected. Potassium leuco phenothiazone sulphate is a possible structure for the conjugate.

The author obtained the following analytical data:

N = 4.10, 4.14%. K = 9.4, 9.6%. Na = 0.90, 0.96%. SO_3 = 20.81, 20.97%.

The unhydrolysed conjugate gave a strong reduction with the Folin-Ciocalteu phenol reagent; the intensity of the reaction was not increased by hydrolysis.

Examination of the precipitate obtained on hydrolysis of the conjugate showed that it was appreciably soluble in hot water and in strong acids or alkalies. Thus, if a very dilute solution of conjugate was acidified, no precipitate formed. Phenothiazine itself is highly insoluble under these conditions. An alcoholic solution of the substance when poured into a small volume of water gave a greyish precipitate which showed an appreciable solubility, thus differentiating it from phenothiazine.

Treatment of the substance with sodium hydroxide and hydrogen peroxide resulted in the immediate appearance of an intense red colour; phenothiazine is only slowly oxidized under these conditions. This suggested that the precipitate from the hydrolysis of the conjugate is the leuco form of either phenothiazone or thionol. If a fairly strong solution of the substance in sodium hydroxide was treated with hydrogen peroxide, the red oxidation product precipitated out. This indicated the possibility of phenothiazone, rather than thionol, as the former is stated by Bernthsen (1) to be insoluble in alkali, the latter soluble.

A sample of this substance, recrystallized from dilute alcohol, was analysed by Professor Young, who reported the values for carbon and hydrogen given below. Total nitrogen was determined by the author.

Precipitate, found:	C = 64.0%;	H = 4.46%;	N = 6.71%.
Leuco phenothiazone, calculated:	C = 66.9%;	H = 4.22%;	N = 6.51%.
Phenothiazine, calculated:	C = 72.3%;	H = 4.55%;	N = 7.03%.

These analyses, together with the properties described, indicate that the compound is probably impure leuco phenothiazone, and the conjugate the potassium salt of its ethereal sulphate. The analysis of the conjugate is summarized below, the calculated values being based on the formula $C_{12}H_8NSOSO_3K$ = 333.3.

C,	found = 43.2%;	calculated = 43.2%
H,	found = 2.72%,	calculated = 2.42%
N,	found = 4.12%;	calculated = 4.20%
Ash,	found = 23.85%;	calculated = 26.13%
K,	found = 9.5%;	calculated = 11.73%
Na,	found = 0.93%;	calculated = —
SO_3,	found = 20.9%;	calculated = 24.0%
Leuco phenothiazone (colorimetric),	found = 64.3%;	calculated = 64.6%

Blood

Blood serum showed only a trace of red colour on exposure to the atmosphere, but treatment with strong acid (sufficient to redissolve the protein) at room

temperature gave a deep mauve colour. This colour was slightly intensified by hydrogen peroxide but not by heating. The pigment was not extractable by chloroform, probably owing to combination with the protein. It was concluded that a conjugate is present in blood serum, presumably the same as that excreted in the urine.

Addition of *p*-toluenesulphonic acid to the serum precipitated the protein and hydrolysed the conjugate. It was demonstrated by the addition of this reagent to washed and haemolysed erythrocytes that they contained no conjugate.

Milk

The milk of lactating ewes treated with phenothiazine turned a definite pink on exposure to the air. Acidification with strong hydrochloric or *p*-toluenesulphonic acid resulted in a strong mauve colour, indicating the presence of the conjugate, as in blood and urine. Being water soluble, it was absent from the fat globules.

Quantitative Examination

An outline of the quantitative methods applied to the faeces, urine, blood, and milk of treated sheep is presented here. Results obtained by use of these methods are given in the following paper (9), where it is demonstrated that about one-half of the phenothiazine remains unabsorbed, and most of the remainder is recovered in the urine.

The colorimetric analyses were performed with the Klett-Summerson photoelectric colorimeter and the green filter No. 54. Since the colour intensities in the acid solutions did not strictly follow Beer's law, unknowns and standards of similar concentration were compared simultaneously. Alternatively, a calibration curve could be used. The leuco phenothiazone used as standard was prepared by recrystallization from alcohol of the precipitate obtained on hydrolysis of the urinary conjugate.

Faeces

The faeces were collected, dried at 110° C., ground and mixed thoroughly. The unchanged phenothiazine was determined by the following modification of the method used by Eddy and DeEds (3) for phenothiazine in spray-residues on fruit. Samples weighing 1 gm. were extracted by boiling with successive portions of 10, 10, and 5 ml. of 95% ethanol, the extracts being filtered, cooled and made up to 25 ml. Aliquots containing about 0.1 mg. of phenothiazine (as determined by preliminary tests) were transferred to a colorimeter tube graduated at 5 ml., and diluted to 2 ml. with 95% ethanol. About five drops of saturated bromine water was added and the excess bromine removed by boiling in the water bath. The solution was cooled, made up to the 5 ml. mark with 95% ethanol, and read in the colorimeter against a standard of 0.1 mg. of recrystallized phenothiazine in alcohol, similarly treated.

The blanks to be deducted from the observed readings of the unknowns were prepared from normal faeces similarly treated, and diluted to an equal extent. The alcoholic extracts of the faeces are a dark green colour, which disappears on treatment with bromine. For this reason, the extracts of faeces containing phenothiazine could not be used for blanks. By this method, phenothiazine added to normal faeces could be recovered quantitatively.

Blood Serum

Various protein precipitants were tested and found to be unsuitable. Marshall (6) has used *p*-toluenesulphonic acid for the determination of sulphanilamide and its conjugate, and it was found suitable for the estimation of the leuco phenothiazone conjugate when used in very high concentration. Addition of 3 ml. of 30% *p*-toluenesulphonic acid to 2 ml. of serum, followed by a drop of 6% hydrogen peroxide to insure complete oxidation, gave maximal colour; heating did not increase the colour.

However, equally satisfactory results were obtained by the use of excess hydrochloric acid without precipitation of the proteins. To 2 ml. of serum in the colorimeter tube was added 3 ml. of concentrated hydrochloric acid and one drop of 6% hydrogen peroxide. The colour was compared with a standard of leuco phenothiazone similarly treated. (0.002% leuco phenothiazone gave a reading of 345.)

Serum diluted to an equal degree with water provided a suitable blank, as determined by addition of conjugate to normal serum: quantitative recovery was thus obtained. Even badly haemolysed serum could be analysed by this means, as verified by suitable controls.

Urine

The urine was heated above body temperature to dissolve precipitated conjugate, filtered, and diluted to a suitable extent as indicated by preliminary tests. To two volumes of diluted urine were added three volumes of concentrated hydrochloric acid and a drop of 6% hydrogen peroxide. The colour intensity was read in the colorimeter against a leuco phenothiazone standard, as before. The blank was 7 *N* hydrochloric acid. This method gives total leuco phenothiazone. Free leuco phenothiazone may be determined by oxidation with hydrogen peroxide, and comparison with a standard aqueous phenothiazone.

Milk

Milk was centrifuged and the defatted liquid used for analysis. Addition of hydrochloric acid developed the typical red colour but the solution was too turbid for colorimetric reading. Finally it was found that addition of three volumes of 30% *p*-toluenesulphonic acid to two volumes of milk gave a clear filtrate which could be read, after addition of a drop of 6% hydrogen peroxide. The values thus obtained for the concentration of conjugate in the milk were compared with those obtained from blood samples taken simultaneously.

Discussion

As indicated in the following paper, about one-half of the phenothiazine administered to sheep was excreted unchanged in the faeces. A major portion of the balance was recovered in the urine, having been oxidized, absorbed, and conjugated.

The site and mode of absorption are at present unknown. Absence of oxidation products from the faeces suggests either that phenothiazine is absorbed as such, or that it is oxidized in the gastrointestinal tract and immediately absorbed. Since phenothiazine is highly insoluble, the latter alternative seems more probable.

Phenothiazone appears to result from mild oxidizing conditions; according to Bernthsen (1) it is formed by oxidation with atmospheric oxygen, especially in the presence of alkali. It is therefore surmised that a portion of the phenothiazine is oxidized in the gastrointestinal tract to phenothiazone or its leuco form; this is promptly absorbed and the major portion is conjugated to ethereal sulphate and excreted through the urinary tract.

DeEds *et al.* (2) believe that phenothiazone is too insoluble to act as an intermediate in phenothiazine excretion. However, its solubility is as great as that of thionol; the author estimated a solubility of about 0.01% at room temperature. Gersdorff and Claborn (4) used a 0.005% solution in their experiments, and Goldsworthy and Green (5) give the solubility as 0.004%. It is of interest to note that the latter authors believe phenothiazone to be the derivative possessed of fungicidal activity.

The findings with sheep differ from those of DeEds *et al.* with rats, rabbits, and human subjects, in that the phenothiazine seems to be excreted in the urine almost entirely in the form of the ethereal sulphate of leuco phenothiazone. No evidence was found for the presence of a conjugate of phenothiazine. Whether any of the free pigment in the urine is thionol was not tested; in any case it would be only a small fraction of the total excretory products.

In view of the confusion regarding the properties of these various derivatives of phenothiazine that occur in the animal body, it is felt that there is need for a more thorough investigation of their chemistry and pharmacology.

Acknowledgment

The author is indebted to Prof. Leslie Young, of the Department of Biochemistry, University of Toronto, for many valuable suggestions, and for the analyses performed in his laboratory.

NOTE. After the manuscript of this paper went to press it was found that liver catalase may be completely inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazine or phenothiazone. (It was also inhibited by sulphapyridine and by hexylresorcinol, but not by sulphanilamide.) This offers an explanation of the vermicidal and bactericidal

action of these compounds. The inhibition is probably due to the phenolic hydroxyl: leuco phenothiazone, leuco thionol, and thionol all reduced Folin's phenol reagent, but phenothiazone did not. The leuco compound isolated from the urinary conjugate and oxidized by aeration did not reduce the phenol reagent nor inhibit catalase, these observations affording further evidence that it is leuco phenothiazone.

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STUDIES ON EFFECTS AND EXCRETION OF PHENOTHIAZINE WHEN USED AS AN ANTHELMINTIC FOR SHEEP[1]

By W. E. Swales[2] and H. B. Collier[3]

Abstract

Tests to determine the effects and excretion of phenothiazine and derivatives have been made on young sheep, barren ewes, pregnant ewes, and lactating ewes. Following the development of adequate technical methods, over 80% of the total dose was recovered from the faeces and urine in roughly equal amounts. Observations were made upon the occurrence of the maximum concentrations of total leuco phenothiazone in the blood, urine, and milk. No definite toxic effect has been noted. Further evidence of anthelmintic efficiency of phenothiazine is presented. The bacteriostatic effect of the derivatives in the milk is noted.

Eddy *et al.* (4), in a second publication on "Studies on phenothiazine," noted that a dye substance was excreted in the urine of white rats when these animals were dosed with phenothiazine. DeEds *et al.* (2, 3) and Thomas *et al.* (8), later made detailed studies of the nature and effects of the derivatives excreted through the urinary tract of rats, rabbits, and man. Harwood *et al.* (5) noted the red-stained urine from swine treated with phenothiazine, and listed this effect, as well as constipation, as host reactions to the drug. In the treatment of sheep with phenothiazine the excretion of the dye in the urine is an outstanding and somewhat objectionable characteristic.

During the studies on the anthelmintic effect of this chemical in sheep, observations have been made upon the effect and excretion of phenothiazine when administered in the form of disintegrating tablets as described by Swales (6, 7). Collier (1) undertook a study of phenothiazine and derivatives as they appeared in the blood, urine, faeces, and milk. The present study is a series of observations made upon animals after they had received therapeutic doses of commercial phenothiazine of approximately 95% purity, in the form of disintegrating tablets. Owing to the necessity of using this anthelmintic drug during the early spring months before the animals go to pasture, observations on pregnant and lactating ewes were of importance.

Technique

The collection of faeces was made by fitting a leather harness on the animal in such a way that one band encircled the neck, one the thorax and another the posterior abdomen; these bands were connected by a strap along the middle of the back and one along each side. Snaps were fitted on the posterior band and on the two side straps in such a way that a canvas bag, made to

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cover the hindquarters, could be easily attached and detached. By the use of this apparatus all the faeces were collected at intervals during an experiment.

Urine was obtained by enclosing the animal in an elevated pen and by attaching a rubber bag over the preputial orifice; a rubber tube leading from the base of the bag, through the pen floor and into a glass container, facilitated frequent collection.

The animal's head was held in a stanchion at the front of the pen, thus making the collection of peripheral blood from ear veins a simple matter. In collecting a sample of from 10 to 15 cc. the severance of a marginal branch of the greater auricular vein was found most satisfactory; the same incision could be used for several collections by simple manipulation.

The qualitative and quantitative determinations of derivatives were made by methods described by one of us (Collier (1)).

Preliminary Observations

The first observations were qualitative. During critical testing of the anthelmintic effect of phenothiazine tablets, particles of commercial phenothiazine were repeatedly found on microscopical examination of faeces passed between the 8th and 12th hours after treatment; such particles became more numerous during the next 12 hr. At necropsy particles were also found present in the stomachs and intestines four days after treatment. The urine passed between the first hour and the fourth day after treatment rapidly turned red on exposure to air. Tests of the blood on the 8th, 12th, 17th, and 24th hours after treatment of two animals revealed the presence of derivatives of phenothiazine. Tests were then planned in order to determine the relative amounts of the original dose that were excreted at various intervals through the intestinal and urinary tracts.

EXPERIMENT I (Jan. 29 to Feb. 7, 1940)

Subject: Ram lamb, weighing 62 lb.

Treatment: 31 gm. tablet formula (=23.6 gm. pure phenothiazine).
No fasting before or after treatment.

Analyses:

Blood	Total leuco phenothiazone.		
	Positive in	1½	hr. after treatment.
	Maximum in	7½	hr. after treatment.
	Negative in	72	hr. after treatment.
Urine	Total leuco phenothiazone.		
	Positive in	1½	hr. after treatment.
	Maximum in	6	hr. after treatment.
	Negative in	5	days after treatment.

Faeces not analysed; blood and urine quantitative estimations not accurate owing to imperfect methods. The efficiency against *Ostertagia* and *Oesophagostomum* was 100%, the former being all discharged in the faeces in the first 24 hr. and the latter in 48 hr. At necropsy no sign of toxic effect could be detected by macroscopic examination; the kidneys were examined for evidence of concretions of conjugate in the tubules, with negative results.

EXPERIMENT II (Feb. 7 to 13, 1940)

Subject: Male, aged 2 years, weighing 123½ lb.

Treatment: 61.8 gm. tablet formula (=47.0 gm. pure phenothiazine). No fasting.

Analyses:

—	Post-treatment times, hr.	Total leuco phenothiazone, %
Blood serum	1	0.0016
	5	0.0053
	9	0.0074
	24	0.0057
	48	0.0030
	72	0.0025

Urine: Method not sufficiently accurate for records of quantitative determinations. Urine was positive after 1½ hr. and was still positive when the experiment was discontinued three days later.

Faeces: Method not accurate, but over 50% of the original dose of phenothiazine appeared to be present in the faeces passed during the first four days. The maximum was reached during the second day and the faeces collected on the fourth day were practically negative.

This animal was not sacrificed, so no accurate estimation of anthelmintic efficiency was made. A simple sugar flotation test of 15 gm. of faeces revealed only five strongyloid ova prior to treatment; a similar post-treatment test was negative.

EXPERIMENT III (Feb. 13 to 17, 1940)

Subject: Male, aged 10 months, weighing 115 lb.
Pure infection with *Ostertagia circumcincta*, 800 ova per gm. of faeces.

Treatment: 28.8 gm. tablet formula (=21.9 gm. pure phenothiazine, or 0.19 gm. per pound body weight). Half the dose used in Experiment II per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	1½	0.0012
	5	0.0033
	24	0.0042
	48	0.0009
	72	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	5 - 24	523	0.63	3.29	173
	24 - 48	437	1.18	5.17	215
	48 - 72	435	0.25	1.10	46
	72 - 93	—	Trace	—	—
	93 -	—	Zero	—	—
			Total	9.56	

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	gm.	mg./hr.
	0 - 1¼	48	0.036	0.017	14
	1¼ - 4	75	0.215	0.161	59
	4 - 5	70	0.472	0.330	330
	5 - 21	450	0.669	3.010	188
	21 - 29	375	0.653	2.450	306
	29 - 45	450	0.431	1.940	121
	45 - 69	675	0.138	0.930	39
	69 - 93	435	0.042	0.183	8
			Total	9.02 (8.4 gm. phenothiazine)	

Recovery of Phenothiazine

Faeces	9.56 gm. =	43.6%
Urine	8.40 gm. =	38.4%
Total	17.96 gm.	82.0% recovery

Post-treatment Tests of Faeces for Residual Infection

Quantitative tests (Stoll technique) were negative on the first day after treatment. By methods of concentration and flotation in sugar solution, 47 ova of *Ostertagia* were recovered from 15 gm. of faeces on the first day, but thereafter for 15 days the faeces were free of ova. On the following 36 days (March 1 to April 5, 1940) from one to six ova were recovered from 15 gm. of faeces, but the concentration tests then became and remained negative. At the time of treatment the infection was of 63 days' duration, having been induced by the administration of 75,000 infective larvae of *O. circumcincta*.

EXPERIMENT IV (March 7 to 12, 1940)

Subject: Male, aged 10½ months, weighing 113 lb.
Pure infection with *O. circumcincta*, 400 ova per gm. faeces.

Treatment: 56.0 gm. tablet formula (=42.5 gm. pure phenothiazine, or 0.38 gm. per lb. body weight).
Double the dosage of Experiment III per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	6	0.0072
	12	0.0048
	24	0.0034
	48	0.0024
	72	0.0004
	96	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	8 - 24	41	1.71	0.70	44
	24 - 48	460	3.75	17.30	720
	48 - 72	395	0.04	0.16	7
	72 - 96	375	0.54	2.02	84
	96 -103	87	0.014	0.012	1.7
	103 -			Zero	0
			Total	20.2 gm.	

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	gm.	mg./hr.
	0 - ½	35	0.014	0.005	10
	½- 4	185	0.493	0.913	261
	4 - 6½	193	0.580	1.120	448
	6½- 12	320	0.498	1.595	290
	12 - 24	600	0.770	4.620	385
	24 - 48	815	0.610	4.960	207
	48 - 72	710	0.256	1.815	76
	72 - 96	1400	0.046	0.642	27
	96 -102	610	0.015	0.092	15
	102 -	—	Zero	—	0
			Total	15.8 (14.6 gm. phenothiazine)	

Recovery of Phenothiazine

Faeces	20.2 gm.	=	47.5%
Urine	14.6 gm.	=	34.3%
Total	34.8 gm.	=	81.8% recovery

Post-treatment Tests of Faeces

Qualitative tests were negative on the day following treatment, and remained so for the following four weeks.

Discussion of Experiments I to IV

The above mentioned experiments have shown that between one-third and one-half of the total dose is excreted through the urinary tract. The early appearance (one-half hour) of derivatives in the blood and urine indicates that very rapid oxidation, absorption, and conjugation take place in the upper gastro-intestinal tract. In Experiment IV it will be noted that the sheep was constipated at the time of dosing and that this condition persisted for the first 24 hr. This temporary constipation delayed the faecal output of phenothiazine, but had no apparent effect upon the blood and urine levels of total leuco phenothiazone. However, if the constipation had not been corrected by the phenolphthalein content of the formula it is possible that a larger proportion of the dose would have been absorbed and excreted through the urinary tract, and that less phenothiazine would have been available for anthelmintic action in the lower intestines. This hypothesis constitutes the rationale for continuing the use of relatively large doses of the drug accompanied by a laxative, if high efficiency is to be maintained.

The maximum concentration of derivatives occurred in the blood and urine after about six hours, presumably when the particles of phenothiazine had spread throughout the gastro-intestinal tract. The blood and urine curves were almost parallel, indicating that excretion closely follows absorption.

The blood levels almost certainly indicate the intensity of absorption. In Experiment III the dosage was reduced to half that used in Experiments II and IV, and the concentration in the blood was noted to be proportionately reduced. However, it is of interest to note that the same fraction of the total dose was absorbed in Experiments III and IV.

The maximum faecal excretion occurred later than the maximum in the urine. The urine probably remains positive as long as particles of phenothiazine are present in the gastro-intestinal tract but the small amounts of blood conjugate are not detectable in the later stages. However, the possibility of concretions of the conjugate forming in the urinary tract must not be overlooked, even although such an occurrence has not been noted during the experimental work on this drug.

The high concentration of leuco phenothiazone in the urine of sheep, leading to rapid oxidation to an intensely red dye substance on exposure to air, constitutes an objectionable feature in the practical use of the drug. In the case of sheep used for commercial wool production, steps must always be taken to avoid urine contamination of wool for four days after treatment; prior shearing or the use of an abundance of pervious bedding are suggested.

Experiments III and IV have produced further evidence to show the complete efficacy of this treatment against *Ostertagia*, even with a very much reduced dosage.

The Treatment of Breeding Animals and Excretion of Phenothiazine Derivatives in the Milk

Previous tests having shown that phenothiazine, administered in the described manner, did not result in any discernible toxic effect on young animals and barren females, the question arose as to possible effects upon pregnant ewes, lactating ewes, and lambs nursed by treated ewes.

Experiment V

A preliminary test was conducted in August 1939, on two ewes, one weighing 137 lb., and nursing eight-weeks-old twin lambs, the other weighing 126 lb. and nursing one lamb of the same age. The ewes received 51.4 gm. and 47.2 gm. respectively of the tablet formula (= 0.28 gm. per lb. body weight of pure phenothiazine).

The anthelmintic effect was good, the egg counts dropping from 600 and 200 ova per gm. to a total recovery from 15 gm. of 2 and 3 ova respectively. No effect on the lambs could be detected.

Experiment VI

Further tests were made in March 1940, as presented in Table I.

TABLE I

HISTORIES OF PREGNANT OR LACTATING EWES TREATED WITH PHENOTHIAZINE

Ewe	Treatment (gm. tablet formula = 80% commercial phenothiazine)	Date	Parturition	Interval	Lambs
(1) Pregnant	50	15 March	27 March	12 days	1 normal
(2) Pregnant	50	15 March	21 March	6 days	2 normal
(3) Pregnant	56	18 March	3 April	16 days	2 normal
(4) Pregnant	60	19 March	20 March	1 day	2 still-born (severe dystokia)
(5) Lactating	50	1 April	27 March	—	2, 1 still-born 1 normal
(6) Lactating	55	11 April	21 March	—	3, 1 still-born 2 normal
(7) Lactating	55	11 April	23 March	—	2 normal
(8) Lactating	Control	—	26 March	—	2 normal

It is possible that the laxative effect of the treatment may have accelerated the oncome of parturition in the case of Ewe No. 4, and may have been a contributing factor in the loss of the two foetuses. Blood was obtained from one foetus and was found to be free of leuco phenothiazone.

No adverse effect upon the other ewes and lambs was noted, all the lambs being very thrifty.

Ewe No. 4 was then used as a subject for further studies on the presence of derivatives of phenothiazine in the milk. It was noted that the milk turned to a pink colour after being exposed to air and light for several hours, and that it did not decompose when left standing in a warm room for several days.

EXPERIMENT VII (March 26 to 30, 1940)

Subject: Lactating Ewe (No. 4).

Treatment: 50 gm. tablet formula (= 38 gm. pure phenothiazine).

Analyses:

Post-treatment times, hr.	Total leuco phenothiazone, %	
	Blood serum	Milk
4½	0.0056	0.0029
11	0.0054	0.0090
24	0.0025	0.0140
48	Trace	0.0045
55	—	0.0024
72	Neg.	0.0006
80	Neg.	Trace
96	Neg.	Neg.

At each collection all the available milk was removed from the udder.

EXPERIMENT VIII

Subject: As in Experiment VII.

Treatment: As in Experiment VII.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	6½	0.0098
	24	0.0044
	48	0.0010
	72	Zero

Milk	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	mg.	mg./hr.
	0 – 1	13	0	0	0
	1 – 2	21	0.0008	0.2	0.2
	2 – 3	19	0.0026	0.5	0.5
	3 – 6	57	0.0112	6.4	2.1
	6 – 8½	40	0.0140	5.6	2.2
	8½ – 24	315	0.0145	45.7	3.0
	24 – 48	540	0.0038	20.5	0.9
	48 – 56	200	0.0005	1.0	0.1
	56 – 72	420	0	0	0
			Total	79.9 mg. (0.21% of dose)	

Experiment IX (April 1 to 3, 1940)

This experiment was conducted in order to find if milk withdrawn normally by a lamb would continue to be positive for leuco phenothiazone for a period comparable with those in Experiment VII. The test was qualitative, simple acidification by two parts of hydrochloric acid being used to produce a colour reaction.

Subject: Lactating Ewe (No. 5).

Treatment: 50 gm. tablet formula (38 gm. pure phenothiazine).

Post-treatment times, hr.	Test for leuco phenothiazone
2	Positive
18	Strongly positive
20	Strongly positive
24	Strongly positive
26	Strongly positive
42	Trace
45	Negative

This experiment appears to indicate that when the milk is completely withdrawn by a normal lamb the leuco phenothiazone content may more closely parallel that in the blood than is indicated in the two previous experiments.

Discussion of the Studies in Milk

The above mentioned experiments show that, although derivatives of phenothiazine are excreted in the milk of lactating ewes, the total amount of such derivatives is too small to be of any significance in so far as direct effect upon the lamb is concerned. The evident bacteriostatic effect of the derivatives in the milk may be of interest, and warrants further study.

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CANADIAN WILTSHIRE BACON

XII. EFFECT OF HEAT TREATMENT ON THE COLOUR AND COLOUR STABILITY OF BACON[1]

BY C. A. WINKLER[2] AND J. W. HOPKINS[3]

Abstract

The colour of quadruplicate pieces of factory-cured bacon heated at temperatures of 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr. was measured at the conclusion of heating, and also after subsequent exposure of the samples for 12, 20, and 96 hr. at 10° C. and 95% relative humidity. Interacting effects of time and temperature of heating on colour at the conclusion of treatment were demonstrable. At 40 and 50°, total intensity increased with the duration of heating; at 60 and 70°, there was no definite trend, and at 80° it diminished markedly as the period of heating was prolonged. The maximum average intensity resulted from heating at 70°.

The decrease in intensity after 12 hr. exposure was related to the duration, but not to the temperature, of previous heating. There was no significant change in intensity between 12 and 20 hr., but a further decrease was evident at the end of 96 hr. The decrease in green intensity was still related to the duration, rather than to the temperature, of heat treatment, but the effects of duration of heating on red and blue stability, noted at 12 and 20 hr., were now replaced by temperature effects.

Partial correlation coefficients indicate that increased nitrite content of the meat at the conclusion of heating tended to be associated with a lower intensity of colour. On the other hand, both nitrite content and loss in weight (chiefly moisture) on heating were correlated with increased colour stability on exposure.

In the preceding paper of this series (2), White, Cook, and Winkler describe the details of an experiment designed to investigate the effect of heating Wiltshire bacon, as in smoking, for various periods at different temperatures, on its nitrite content and colour. The experiment comprised four periods of heating, namely, 5, 10, 20, and 40 hr., and six temperatures, 20, 40, 50, 60, 70, and 80° C., or 24 treatments in all. Four out of 16 "backs" cut from individual factory-cured sides were allotted at random to each of the four periods of heating, and each back was subdivided into six portions which were allocated, also by a random process, to the six temperatures maintained for each period of time. The above-mentioned authors conclude that heating at 55° C. resulted in an approximately normal nitrite content. Temperatures

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below 55° resulted in increased amounts of nitrite being found on analysis, whereas higher temperatures diminished the concentration of this constituent. These effects of temperature were accentuated by prolonging the time of heating.

It is now desired to describe the results of colour measurements of the 96 individual samples after treatment, secured by means of the photoelectric comparator employed in other phases of this investigation, already described elsewhere (3). The effect of heat treatment on colour and colour stability will first be dealt with, after which the correlation between these colour measurements and the foregoing determinations of nitrite content will be discussed.

Effect of Heat Treatment on Colour

The photoelectric comparator was employed as before to determine the component intensities of red, green, and blue light, relative to the white standard, reflected or scattered from the surface of the cut samples (4), and the sum of these three relative values was again taken as the index of colour intensity or total brightness.

Intensity or Total Brightness

Table I shows the results of an analysis of the variance (1) of the colour intensity as defined above, at the conclusion of heating of the 80 individual samples treated at temperatures of 40 to 80° C., and Table II the treatment averages, as well as the means by periods, for all temperatures, and by temperatures, for all periods. As was explained by White, Cook, and Winkler (2) the effect of temperature, which is deduced from comparisons between subsamples of the same sides, is more accurately determined than that of period of heating, which is affected by the variability between sides. This is reflected in the magnitude of the mean square errors *(a)* and *(b)* in Table I.

The analysis of variance demonstrates a highly significant effect of temperature on subsequent intensity of colour, and reference to Table II will indicate that the mean intensity, averaged for all periods, increased with

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR TOTAL BRIGHTNESS OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square
Period of heating (all temps.)	3	261.0
Error *(a)* (between sides)	12	303.8
Temperature (all periods)	4	3,273.0***
Interaction, temp. × period	12	618.6***
Error *(b)* (within sides)	45	39.0

****Exceeds mean square error, 0.1% level of significance.*

TABLE II

Average colour intensity or total brightness of bacon samples at conclusion of heat treatment

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	121	120	126	154	163	168	146
10	115	115	134	155	163	158	145
20	113	128	139	152	157	143	144
40	—	143	158	161	169	128	152
Mean	116	126	139	156	163	149	147

temperature from 40 to 70°, falling off again however at 80°. The means for the four periods of heating, when taken over all five temperatures, do not differ significantly, but there is a significant interaction of temperature and duration of heating. Thus at 40 and 50°, the samples exhibited an increasing intensity of colour as the duration of treatment was prolonged. At 60 and 70° there was no appreciable trend, and at 80° the trend was the reverse of that noted at 40 and 50°, the brightness diminishing markedly with length of heating at this temperature.

For the reasons previously stated (2), the observations made on the samples heated at 20° C. were not included in the foregoing analysis of variance. A separate examination of these data did not indicate any significant difference in the brightness of the samples held for 5, 10, and 20 hr. at this temperature.

Chroma or Colour Quality

Tables III, IVA, IVB, and IVC give the analyses of variance and average values of the component red, green, and blue intensities of the samples subjected to the various treatments, in the same form as that adopted in Tables I and II above.

TABLE III

Analysis of variance of component intensities of colour of bacon samples at conclusion of heat treatment

Variance due to	Degrees of freedom	Mean square		
		Red	Green	Blue
Period of heating (all temps.)	3	39.7	50.5	30.3
Error *(a)* (between sides)	12	29.9	33.3	28.9
Temperature (all periods)	4	558.3***	336.2***	404.1***
Interaction, temperature × period	12	94.5***	61.7***	15.5
Error *(b)* (within sides)	45	9.6	5.1	11.6

****Exceeds mean square error, 0.1% level of significance.*

TABLE IV. A

AVERAGE INTENSITY OF RED COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	49.0	46.0	49.0	61.0	64.9	65.6	57.3
10	46.4	46.0	53.4	62.7	65.7	61.4	57.9
20	43.7	50.8	53.9	61.0	62.4	57.8	57.2
40	—	57.2	62.7	63.8	66.5	50.9	60.2
Mean	46.3	50.0	54.8	62.1	64.9	59.0	58.2

The analyses of variance of red and green, and the treatment averages shown in Tables IVA and IVB, both reproduce features already noted in the results for total brightness, namely an increase in the average intensity for all periods of treatment with temperature up to 70°, followed by a reduction at 80°, and a significant interaction of temperature and treatment, intensity of colour increasing with duration of heating at 40 and 50°, and decreasing at 80°. There might seem to be some difference in the behaviour of the blue component, for its interaction mean square in Table III does not attain

TABLE IV. B

AVERAGE INTENSITY OF GREEN COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	37.9	37.3	39.3	46.1	50.0	52.6	45.1
10	35.4	35.2	41.2	47.5	50.4	48.7	44.6
20	35.4	39.4	42.0	46.7	48.9	44.7	44.4
40	—	44.2	49.4	51.1	54.2	40.0	47.8
Mean	36.2	39.0	43.0	47.9	50.9	46.5	45.4

significance. Reference to Table IVC will, however, indicate that there is in fact some reversal of the time effect at the high and low temperatures, but that the effects of treatment in general are less pronounced in this component, and that the differences between the intensities recorded after heating for 5 and 10 hr., and between those for 20 and 40 hr., in particular, are for the most part quite insignificant. These comparisons, which contribute eight of the total 12 degrees of freedom for interaction, dilute the variance computed from the data as a whole, and when the remaining four degrees of freedom, representing the average interaction, with temperature, of heating for 5 and 10 hr. on the one hand and for 20 and 40 hr. on the other were isolated, they

TABLE IV. C

AVERAGE INTENSITY OF BLUE COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	33.8	36.4	37.4	47.0	47.9	49.6	43.6
10	33.3	33.8	39.7	44.9	46.6	47.4	42.5
20	34.3	37.4	42.8	44.3	46.1	35.5	41.2
40	—	42.2	45.8	46.0	48.5	37.0	43.9
Mean	33.8	37.4	41.4	45.5	47.3	42.4	42.8

yielded a mean square of 34.9, significantly in excess of the mean square error *(b)*, 11.6. It is to be concluded, therefore, that the treatments imposed influenced all three colour components in a similar manner, but that the magnitude of the effects was greatest in the red and least in the blue region.

The samples maintained at 20° C., which were again considered separately, failed to exhibit any statistically significant differences attributable to the period of heating.

Effect of Heat Treatment on Colour Stability

In addition to the foregoing observations at the conclusion of heat treatment, further measurements of the intensity of the red, green, and blue components of colour were made after the treated samples of bacon had been exposed in a light-proof chamber for 12, 20, and 96 hr., at 10° C. and 95% relative humidity. These enabled the effects of heat treatment on colour stability, which will again be considered under the separate heads of total intensity and colour quality, to be followed in some detail.

Intensity or Total Brightness

Tables V and VI summarize the results of the observations of change in total intensity as defined above, the former giving the analyses of variance (40 to 80°), and the latter the treatment averages for the 12-, 20-, and 96-hr. periods of exposure.

It will be noted at once from Table V that whereas the change in intensity after both 12 and 20 hr. exposure varied significantly in relation to the duration, but not the temperature, of heating, after 96 hr. these effects of heating period had disappeared. Curiously enough, the samples heated for the shortest periods showed the greatest decreases in intensity of colour after 12 hr. exposure (Table VI). There was no significant change in intensity between 12 and 20 hr.; in fact, the mean decrease observed after 20 hr., 5.2 units, was slightly less than the corresponding figure of 6.3 after 12 hr., but the difference is within the limits of the experimental error. By 96 hr., however, the mean decrease (at 19.0 units) was appreciably greater and, as noted above,

TABLE V

ANALYSIS OF VARIANCE OF CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	245.6***	323.3*	182.0
Error *(a)* (between sides)	12	18.9	58.7	60.8
Temperature (all periods)	4	11.7	9.9	135.6*
Interaction, temperature × period	12	44.0	40.3	43.2
Error *(b)* (within sides)	45	47.3	45.2	45.1

* *Exceeds mean square error, 5% level of significance.*

*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VI

AVERAGE CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−10.6	−11.2	−14.4	−10.0	− 9.4	− 5.2	−10.0
12	10	− 3.2	− 2.4	−12.4	− 9.8	− 7.0	− 7.6	− 7.9
12	20	− 5.9	− 8.2	− 3.6	− 5.3	− 3.6	− 6.1	− 5.4
12	40	—	− 1.2	− 0.8	+ 1.4	− 3.0	− 5.7	− 1.9
12	Mean	− 6.6	− 5.8	− 7.8	− 5.9	− 5.7	− 6.2	− 6.3
20	5	− 7.0	− 7.2	−11.1	− 9.2	− 8.6	− 5.8	− 8.4
20	10	− 0.8	− 2.4	− 9.8	−10.6	− 8.8	− 7.6	− 7.8
20	20	− 2.3	− 5.1	− 1.4	− 7.9	− 3.8	− 7.3	− 5.1
20	40	—	− 0.8	+ 1.3	+ 5.6	− 0.8	− 3.2	+ 0.4
20	Mean	− 3.4	− 3.9	− 5.3	− 5.5	− 5.5	− 6.0	− 5.2
96	5	−15.2	−15.4	−20.7	−22.4	−22.7	−18.1	−19.9
96	10	− 7.9	− 7.6	−19.5	−21.8	−21.4	−22.2	−18.5
96	20	− 9.8	−14.8	−12.2	−18.6	−16.4	−13.7	−15.1
96	40	—	−21.0	−23.2	−22.5	−27.3	−17.8	−22.4
96	Mean	−10.9	−14.7	−18.9	−21.4	−22.0	−17.9	−19.0

the earlier effects of duration of heating had disappeared, but differences related to temperature had developed. These were such that the magnitude of the decrease in intensity increased with temperature of heating from 40 to a maximum at 70°, falling off again at 80°. There is thus a parallelism between the effects of temperature on colour at the conclusion of heating,

and on colour stability, which is worthy of remark in view of the correlation between initial intensity and stability noted elsewhere (5). In contrast to the effects on initial colour, however, there was no demonstrable interacting influence of temperature and duration of heating on stability.

Chroma or Colour Quality

Table VII gives the results of analyses of variance of the change in the component red, green, and blue intensities after exposure of the samples for 12, 20, and 96 hr. The results for the red and blue components resemble those already noted in the foregoing analysis of the changes in total intensity in indicating significant differences in stability after 12 and 20 hr. related to the duration of heating, which were replaced by differences ascribable to temperature at the end of 96 hr. Tables VIII and X further indicate that these effects were similar in nature to the ones observed in total intensity. Both of the components mentioned showed a greater reduction at 12 and 20 hr. following the shorter periods of heating, and a maximum decrease in intensity after 96 hr. in the samples heated at 70°.

TABLE VII

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	*Mean square (red)*			*Mean square (green)*			*Mean square (blue)*		
		12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	26.6*	39.6*	23.1	18.0**	17.3	28.0*	47.6***	64.9**	22.0
Error *(a)* (between sides)	12	5.6	9.9	9.0	2.2	5.6	6.8	3.4	9.4	7.4
Temperature (all periods)	4	4.1	8.7	33.5*	1.7	1.3	5.6	5.8	3.0	18.4*
Interaction, temperature × period	12	12.3	9.5	8.9	5.2	4.9	3.5	4.7	4.7	6.0
Error *(b)* (within sides)	45	7.0	4.9	8.3	6.7	6.6	6.1	3.7	6.5	6.0

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

The changes in the green component, on the other hand (Table IX), followed a somewhat different course, perceptible effects of duration of heating persisting even after exposure of the samples for 96 hr., whereas temperature effects were confined to narrow limits, which did not exceed the experimental error. After 96 hr. exposure, however, the decrease in green intensity of the various samples was no longer inversely proportional to the duration of heating. There was still a progressive diminution in loss of intensity from

TABLE VIII

AVERAGE CHANGE IN RED INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−5.2	−3.2	−5.8	− 4.0	− 4.2	− 0.4	−3.5
12	10	−1.8	−1.0	−4.4	− 5.4	− 3.4	− 1.8	−3.2
12	20	−0.0	−2.6	−0.2	− 1.5	− 0.4	− 2.4	−1.4
12	40	—	−0.0	−0.4	− 0.6	− 2.2	− 3.0	−1.3
12	Mean	−2.3	−1.7	−2.7	− 2.8	− 2.5	− 1.9	−2.3
20	5	−4.1	−1.3	−4.5	− 4.0	− 4.7	− 1.8	−3.3
20	10	−1.6	−2.1	−4.8	− 5.5	− 4.8	− 3.4	−4.1
20	20	+0.6	−2.2	+0.3	− 4.4	− 2.2	− 4.1	−2.5
20	40	—	−0.6	−0.3	+ 0.8	− 1.7	− 2.2	−0.8
20	Mean	−1.7	−1.6	−2.3	− 3.2	− 3.4	− 2.9	−2.7
96	5	−7.7	−5.8	−7.9	− 8.9	− 9.9	− 7.2	−7.9
96	10	−4.4	−4.2	−8.3	−10.5	−10.8	−10.9	−8.9
96	20	−3.4	−7.0	−5.0	− 9.3	− 7.4	− 6.7	−7.1
96	40	—	−8.9	−9.2	− 9.6	−11.9	− 8.2	−9.5
96	Mean	−5.2	−6.5	−7.6	− 9.6	−10.2	− 8.2	−8.4

TABLE IX

AVERAGE CHANGE IN GREEN INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−4.1	−3.9	−3.3	−1.6	−2.5	−2.1	−2.7
12	10	−0.6	−0.4	−3.6	−2.2	−1.9	−1.6	−1.9
12	20	−2.4	−3.2	+0.1	−1.7	−0.6	−1.8	−1.4
12	40	—	−0.5	−0.5	+0.7	−0.6	−1.2	−0.4
12	Mean	−2.4	−2.0	−1.8	−1.2	−1.4	−1.6	−1.6
20	5	−2.6	−2.8	−2.2	−0.5	−0.8	−1.0	−1.5
20	10	+0.6	+0.1	−2.2	−1.8	−1.9	−0.4	−1.2
20	20	−1.4	−1.5	+0.5	−0.8	+0.2	−1.2	−0.6
20	40	—	+0.3	+1.1	+2.2	+0.2	−0.7	+0.6
20	Mean	−1.1	−1.0	−0.7	−0.2	−0.6	−0.8	−0.6
96	5	−5.2	−5.0	−5.7	−4.8	−5.9	−5.0	−5.3
96	10	−2.2	−1.9	−5.0	−5.4	−4.9	−4.1	−4.3
96	20	−3.0	−4.3	−3.0	−4.3	−3.3	−3.0	−3.6
96	40	—	−5.8	−7.0	−6.7	−7.4	−4.5	−6.3
96	Mean	−3.5	−4.3	−5.2	−5.3	−5.4	−4.2	−4.9

TABLE X

AVERAGE CHANGE IN BLUE INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−1.3	−4.2	−5.2	−4.4	−2.7	−2.8	−3.9
12	10	−0.8	−1.0	−4.4	−2.3	−1.7	−4.3	−2.7
12	20	−3.5	−2.4	−3.5	−2.1	−2.6	−1.9	−2.5
12	40	—	−0.7	+0.2	+1.3	−0.3	−1.4	−0.2
12	Mean	−1.8	−2.1	−3.2	−1.9	−1.8	−2.6	−2.3
20	5	−0.2	−3.1	−4.5	−4.8	−3.0	−3.0	−3.7
20	10	+0.1	−0.4	−2.8	−3.4	−2.0	−3.8	−2.5
20	20	−1.5	−1.5	−2.2	−2.6	−1.8	−2.0	−2.0
20	40	—	−0.5	+0.5	+2.5	+0.7	−0.3	+0.6
20	Mean	−0.5	−1.4	−2.2	−2.1	−1.5	−2.3	−1.9
96	5	−2.2	−4.5	−7.1	−8.7	−6.9	−5.8	−6.6
96	10	−1.3	−1.6	−6.2	−5.9	−5.7	−7.1	−5.3
96	20	−3.4	−3.5	−4.2	−5.0	−5.6	−4.0	−4.4
96	40	—	−6.3	−7.2	−6.2	−8.0	−5.0	−6.6
96	Mean	−2.3	−4.0	−6.2	−6.5	−6.6	−5.5	−5.7

the samples heated for 5 hr. to those heated for 20 hr., but, of all samples, the ones heated for 40 hr. now showed the maximum decrease. It is possible that after still further exposure, the decrease in green intensity following the various heat treatments might eventually have conformed to that of the other two components, but a further experiment would be required to test this point.

Correlation of Colour and Colour Stability with Moisture Loss and Nitrite Content

White, Cook, and Winkler having shown (2) that the nitrite content of the various samples was significantly affected by the heat treatment to which they were subjected, it was of interest to determine the extent to which changes in colour were related to the effects of heating on nitrite and moisture contents. These authors point out that the observed loss of weight of the samples on heating was an imperfect criterion of drying, as at the higher temperatures there was also some loss of melted fat which was absorbed by the paper wrapping. In the absence of more reliable data, however, the total loss in weight was of necessity adopted as the only available index of dehydration.

Table XI shows the coefficients of simple correlation between the loss in weight and logarithmic nitrite content (2) on the one hand, and the intensity and subsequent stability of colour on the other. There was a significant positive correlation between loss in weight and the intensity of red and green at the conclusion of heating, and a negative association between nitrite content

TABLE XI

COEFFICIENTS OF SIMPLE CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	r, intensity at conclusion of heating	r, change after exposure		
		12 hr.	20 hr.	96 hr.
Loss in weight × total intensity	+.26*	+.26*	+.10	+.09
Loss in weight × red intensity	+.28**	+.06	−.04	−.19
Loss in weight × green intensity	+.29**	+.16	+.10	+.06
Loss in weight × blue intensity	+.19	+.19	+.12	−.06
Log. nitrite content × total intensity	−.34**	+.07	+.23*	+.25*
Log. nitrite content × red intensity	−.37**	+.19	+.27*	+.27*
Log. nitrite content × green intensity	−.34**	−.10	−.01	+.04
Log. nitrite content × blue intensity	−.29**	+.12	+.09	+.20

*Exceeds 5% point ($r = \pm 0.20$).
**Exceeds 1% point ($r = \pm 0.27$).

and all three colour components, the latter indicating that samples above-average in nitrite content tended to have a lower intensity of colour. When the changes in colour on exposure were considered, the only significant correlations in evidence were those between loss in weight during heating and the change in total intensity after 12 hr. exposure, and between nitrite content and the change in red and in total intensity after 20 and 96 hr. All of these coefficients are positive, indicating that above-average loss in weight and nitrite content were associated with greater stability of colour on exposure.

In this connection, however, it is again necessary to take account of the fact that there was a significant negative correlation between the loss in weight and nitrite content of individual samples, and significant positive correlations between the individual red, green, and blue intensities at the conclusion of heating, as well as between the changes in each of these components in any particular sample on exposure. For this reason, the partial correlation coefficients shown in Table XII were computed.

The partial coefficients for total intensity at the conclusion of heating suggest that the apparent correlation of this quantity with loss in weight (Table XI) was a consequence of the negative association, noted above, between loss in weight and nitrite content, the latter alone significantly affecting total brightness. There does seem to have been a significant relation between loss in weight and the individual intensities of green and blue, but as the correlation was positive in one case and negative in the other, the resultant effect on total intensity was inappreciable. Nitrite content, on the other hand, may be judged to have had a demonstrable effect on total intensity, but apparently no selective action on the individual components of colour.

The partial coefficients also bring out relations between loss in weight and nitrite content, and the change in intensity of colour after 12 hr. exposure, not apparent from Table XI. In this case, there were significant effects not

TABLE XII

COEFFICIENTS OF PARTIAL CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	Independent of	r, intensity at conclusion of heating	r, change after exposure		
			12 hr.	20 hr.	96 hr.
Loss in wt. × total intensity	Log. nitrite content	+.04	+.42**	+.36**	+.37**
Loss in wt. × red intensity	Log. nitrite content, green and blue intensity	−.06	+.30**	+.12	−.13
Loss in wt. × green intensity	Log. nitrite content, red and blue intensity	+.22*	−.28**	−.11	+.11
Loss in wt. × blue intensity	Log. nitrite content, red and green intensity	−.22*	+.39**	+.20	+.07
Log. nitrite content × total intensity	Loss in weight	−.24*	+.35**	+.42**	+.43**
Log. nitrite content × red intensity	Loss in weight, green and blue intensity	−.16	+.45**	+.31**	+.14
Log. nitrite content × green intensity	Loss in weight, red and blue intensity	+.11	−.45**	−.24*	−.13
Log. nitrite content × blue intensity	Loss in weight, red and green intensity	−.05	+.43**	+.19	+.17

**Exceeds 5% point ($r = \pm 0.21$).*
***Exceeds 1% point ($r = \pm 0.27$).*

only on the change in total intensity, but also on the changes in red, green, and blue, each independent of the other two. This is a feature of some interest, for which no explanation seems to be readily forthcoming. It is also to be noted that additional loss in weight and nitrite content were associated with increased stability of red and blue over the 12-hr. period, but with a decreased stability of green.

Separate effects of nitrite content on red and green stability were still detectable in the observations made after 20 hr. exposure, but by 96 hr. these independent effects were no longer distinguishable, although there was still a significant correlation between nitrite content and the change in total intensity. Loss in weight during heating was likewise correlated with the total decrease in intensity up to 96 hr., but in this instance independent effects on the individual components apparently did not persist beyond 12 hr. of exposure. Both loss in weight, which was uncorrelated, and nitrite content, which was negatively correlated with the total intensity of colour at the conclusion of heating, were associated with enhanced colour stability on subsequent exposure.

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CANADIAN WILTSHIRE BACON

XIII. TENDERNESS OF BACON AND EFFECT OF HEAT TREATMENT ON TENDERNESS[1]

By C. A. Winkler[2] and J. W. Hopkins[3]

Abstract

Measurements made on uncooked samples of two factory-cured sides from each of 22 Canadian packing plants indicated significant differences between plants in respect of tenderness. The maximum average cutting force required by the product of an individual plant was more than three times the minimum plant average. The individual results however were fairly uniformly distributed over the observed range of variation, neither the maximum nor the minimum representing an isolated extreme. Partial correlation coefficients indicated a significant influence of pH of both pump and cover pickle, in conjunction with number of "stitches" and days in cure respectively, on tenderness, but no appreciable relation of this property to salt content, moisture content, nitrate content or pH of the meat, or number of days from curing to receipt at the laboratory, was demonstrable.

A second series of observations on samples heated at 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr., as in smoking, demonstrated significant interacting effects of the temperature and duration of heat treatment on tenderness. Excepting one notably anomalous result, the general tendency was for toughness to increase with the prolongation of temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. On the average, the maximum toughness was observed after heating at 50° C.

Introduction

In the course of a study of factors affecting the quality of Canadian Wiltshire bacon (1), determinations of the tenderness of two series of samples were made by means of the apparatus designed for this purpose by one of the authors (6). The two series comprised (i) samples of two factory-cured sides from each of 22 Canadian packing plants, and (ii) samples, of the same origin as in (i), subjected to various heat treatments, as in smoking.

As indicated in a previously published description (6) the apparatus actually provides a measure of toughness rather than of tenderness, namely the work required to cut through a piece of meat of specified width and thickness. The operation of the apparatus was the same as that previously described (6), and the data presented as indices of the cutting force are again the areas under the curve traced by the recording pen of the instrument in each case, corrected for thickness of sample. As these are directly comparable, they may be regarded as "relative work", without conversion into standard energy units. It was however found that over the range encountered in this series,

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[2] *Formerly Biophysicist, Food Storage and Transport Investigations. Present address: Department of Chemistry, McGill University, Montreal.*

[3] *Statistician, National Research Laboratories, Ottawa.*

the cutting force required varied more nearly as the square than as the actual thickness of individual samples. A Least Squares solution, based on 105 observations, actually indicated an average increase of 1.82 in the logarithm of the work done per unit increase in log thickness. This relation was accordingly used, in preference to the proportional factor previously adopted (6), to correct the observations for deviations of the individual test slices from the standard thickness of 5.0 mm.

Tenderness of Commercial Sides

Determinations were made on uncooked specimens from each of the 44 sides in series (i). For this purpose a sample of the necessary size was taken from the ham muscle of each side on arrival at the laboratory, the usual precautions being observed to secure representative material free from obvious striations of connective tissue (6).

After correction for thickness, an analysis of the variance (4) of the 44 resulting determinations indicated that the differences between the averages of the two sides from individual plants were significantly greater than would be expected from the observed variability of sides from the same plant. Table I summarizes the results of these observations, from which it is to be noted that the maximum average cutting force required by the product of an individual plant was as much as three times the minimum plant average. The individual results were however fairly symmetrically distributed over the observed range of variation, neither the maximum nor the minimum being an isolated extreme.

TABLE I

SUMMARY OF MEASUREMENTS OF TOUGHNESS, CORRECTED FOR THICKNESS, ON SAMPLES FROM 44 COMMERCIAL SIDES

Toughness	Relative cutting force required
Maximum	
Individual side	4.2
Plant (average of 2 sides)	3.6
Minimum	
Individual side	0.9
Plant (average of 2 sides)	1.2
Mean	2.4

As the sides from the various plants were not all of the same age when received at the laboratory, it would be incorrect to assume that all of the variation in tenderness of product between plants was ascribable to differences in the curing practices employed. Nevertheless, in view of the relation between tenderness and pH previously observed in samples of raw pork adjusted to

various degrees of acidity and alkalinity by injections of lactic acid or ammonia solutions (6), the effect of pH of pickle (3) on the toughness of the present series of samples was investigated. Omitting four plants for which the analytical data were incomplete, statistically significant correlation coefficients of −0.58 between the cutting force required and the product of pH of pump pickle (mean of two samples) × number of "stitches", and of +0.48 between cutting force and the product of pH of cover pickle × number of days in cure were obtained from the data for the remaining 18. There was no appreciable correlation ($r = -0.09$) between the two acidity indices for the same plant, with the result that the partial correlation of each index with toughness, independent of the other, at −0.61 and +0.52 respectively, did not differ significantly from the simple value. It is to be noted that the average pH of the pump pickles, at 7.6, was on the alkaline side of neutrality, and that of the cover pickles, at 6.6, was on the acid side, but in view of the fact that the pH of the meat itself on receipt at the laboratory, although previously shown to be affected to some extent by the pH of the pickles (3), nevertheless varied only within narrow limits, and could not be demonstrably related to toughness on the number of observations available, the mode of action of the pickles in modifying this last property remains obscure.

Coefficients of correlation between toughness and salt, moisture and nitrate content (2), as well as number of days from curing to receipt at the laboratory, were also computed, but failed to indicate any significant relation.

Effect of Heat Treatment on Tenderness

The heat treatments represented in Series (ii) comprised six temperatures, namely, 20, 40, 50, 60, 70, and 80° C., and four periods of heating, 5, 10, 20, and 40 hr. The experimental procedure, which was designed primarily to study the effect of heat treatment on nitrite content and colour, and has been described in detail elsewhere (5), may be summarized as follows: Sixteen "backs" cut from individual sides were allotted at random, four to each of the four periods of heating, and a piece from each back was allocated, also by a random process, to the six temperatures maintained for each period. The individual pieces, having been trimmed free of the cut portions of the ribs and the layer of back fat, were wrapped first in lightly waxed glassine paper and then in kraft paper, and heated in ovens maintained at the specified temperatures. At the conclusion of heating, the surface layers of each piece were trimmed off prior to the selection of suitable samples for the determination of tenderness.

Table II shows the analysis of variance of the measurements on the individual pieces, and Table III summarizes the treatment averages (means of four pieces). Unfortunately, through an oversight, the pieces which should have been heated at 20° C. for 40 hr. were removed at the end of 20 hr.; and those heated at 80° C. for 40 hr. were dehydrated to such an extent that satisfactory measurements of toughness could not be made. The tests of significance in Table II are therefore deduced from the data for 40 to 70°, in

TABLE II

ANALYSIS OF VARIANCE OF TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS HEAT TREATMENTS (40 TO 70° C.)

Variance due to	Degrees of freedom	Mean square
Period of heating (average for all temps.)	3	0.77
Error *(a)*	12	1.10
Temperature of heating (average for all periods)	3	3.96*
Interaction, temp. × period	9	3.30**
Error *(b)*	36	1.02

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

which region the factorial combination of treatments was complete. Table III however includes all of the available observations.

Owing to the fact that the pieces cut from the same back resembled each other more closely than did those from different backs, the comparisons afforded by the experiment are of two levels of precision, as indicated by mean square errors *(a)* and *(b)* in Table II. Over the temperature range 40 to 70°, the differences between the average toughness after the four periods of heating did not attain statistical significance on the number of observations available. This however was due to a balancing of effects, rather than to an absence of any influence of duration of heating, as, in addition to average temperature effects, a significant interaction of temperature with period of heating is demonstrated in the lower part of this table.

The maximum average toughness, over all four heating periods, was observed after heating at 50° C., but owing to the interaction noted above the maximum for the individual periods varied from 60° for the 5 and 10 hr. periods to 40°

TABLE III

AVERAGE TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS HEAT TREATMENTS

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–70°
	20	40	50	60	70	80	
5	3.05	4.05	3.40	4.25	4.28	3.02	3.99
10	3.02	3.75	3.78	4.40	3.02	2.98	3.74
20	3.63	4.35	4.65	3.75	3.48	1.58	4.06
40	—	3.65	5.85	2.78	2.08	—	3.59
Mean	3.31	3.95	4.42	3.79	3.21	2.52	3.84

for 20 hr. and 50° for 40 hr. With the exception of the anomalous value observed after heating at 40° C. for 40 hr., the general tendency was for toughness to increase with duration of heating at temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. It has to be remembered of course that these results were obtained from small pieces of meat, and that the temperature and time effects noted are therefore unlikely to be directly applicable to the commercial treatment of entire sides. Nevertheless, the bearing of these facts upon the operations of commercial smoking will be appreciated, as temperatures ranging from as low as 40° in the smoking of bacon, to 60° or more in the production of "pre-cooked" hams are employed in practice. The reasons, other than possibly the straightforward removal of moisture, for the increase in toughness with time of heating at temperatures up to 50° are at present unknown, but it is of interest to note that the higher temperatures to which "pre-cooked" products are subjected may be expected to have a definitely tenderizing effect.

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PARTIAL CONGENITAL OCCLUSION OF THE VAGINA IN THE SILVER FOX[1]

BY BENJAMIN KROPP[2]

Abstract

A condition of partially imperforate vagina in the silver fox is described and briefly discussed.

The present report deals with a congenitally anomalous urogenital orifice encountered in the silver fox (*Vulpes fulva fulva*). Two different males had been placed successively with the mature vixen, and each male had failed to impregnate her, owing apparently to failure of the female to receive the male intromittent organ. The owner then sacrificed the animal for its pelt since it was worthless as a breeder, and the carcass, in a frozen state, was turned over to the writer for examination.

The opening into the urogenital canal was found to be covered transversely by a dome-shaped septum that completely covered the external aperture except for a small opening at the extreme left lateral margin (Fig. 1). The

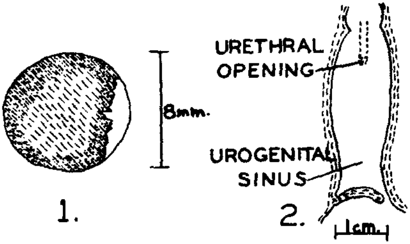

FIG. 1. *Diagrammatic external view of membrane covering urogenital aperture.* FIG. 2. *Diagrammatic frontal section of urogenital sinus and membrane. See text for explanation.*

septal edge bordering the marginal opening was uneven and serrated. The total diameter of the outer aperture in a lateral plane was 0.9 cm., and 0.8 cm. in an antero-posterior plane. The jagged free left edge of the septum

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reached to within 1.5 mm. of the left lateral margin. The membrane was tough and its external surface appeared cornified, although the membrane yielded readily to cutting with scissors. The concavity of the membrane may have been due in part to pressure of the penis during repeated attempts at copulation, but is to be attributed mainly to the effects of post-mortem shrinkage.

Unfortunately, the carcass was not fresh when received so that attempts to determine the nature of the occluding septum microscopically were not undertaken. However, the vagina and associated structures were dissected grossly and the septum found to be separable into two membranous sheets (Fig. 2), the inner sheet continuous with the vaginal lining, the outer sheet continuous with the skin surrounding the opening. Between the two sheets was a very thin layer of loose fibrous material, devoid of blood vessels, which sent small strands into the vaginal musculature. The ovaries, both horns of the uterus, and the fallopian tubes were grossly normal; no corpora lutea were detected in the ovaries. The urinary bladder opened into the urogenital sinus 28 mm. above the membrane, and the opening present in the latter was apparently sufficient to permit escape of urine. It is interesting to note that the presence of the septum could easily have been detected in the living animal if occlusion of the genital tract had been suspected as a possible cause of failure to mate. Removal of the structure with fine scissors could have been effected easily and sacrifice of the animal avoided.

Complete congenital occlusion of the vagina has been frequently encountered in laboratory colonies of adult rats (2) and mice (1). While in these and in other instances the congenital anomaly may vary in form, there is some reason to believe that in rats and mice the condition has an hereditary basis. The condition here described does not seem to be strictly comparable with that in rats and mice. The anomaly is regarded only as a developmental arrest in that the delamination of the vaginal plate failed to continue to completion.

Acknowledgment

The author is indebted for the specimen to Dr. N. V. Freeman.

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EXPERIMENTAL STUDIES ON *STRONGYLOIDES AGOUTII* IN THE GUINEA PIG[1]

By Henry J. Griffiths[2]

Abstract

The suitability and tolerance of the guinea pig to infection with *Strongyloides agoutii* presented an opportunity for the study of the bionomics of this species in an experimental host.

Serial transfer of this nematode through the guinea pig yielded a mixed type (free males and filariform larvae) of free-living development in faecal cultures which occasionally reverted to the indirect mode common to *S. agoutii*. A reversion to the indirect mode of development was produced when ova from faeces of guinea pigs infected with *S. agoutii* were cultured in sterile agouti faeces.

The termination of the prepatent period of *S. agoutii* in the guinea pig was shown to range from 7 to 10 days, and 71% of 58 animals were positive by faecal test by the eighth day. The patent period ranged from three to eight weeks.

The guinea pig was shown to develop an absolute acquired immunity to re-infection with *S. agoutii*. This resistance has been retained over a period of at least 6 to 13 months. An age resistance was not observed in animals one year old and over.

Introduction

Previous studies (10) on the morphology and biology of *Strongyloides agoutii*,* of the golden-rumped agouti *(Dasyprocta agouti)*, led to further investigation on the experimental infection of some common laboratory animals with this species.

For some time it has been known that the infection of certain abnormal hosts with *Strongyloides* may produce radical changes in the mode of free-living development. Brumpt (3) observed an alternation of the free-living cycle when he infected rabbits with *S. papillosus* of sheep. In sheep he observed a great predominance of females in the bisexual generation; in fact, free males were a comparative rarity. However, in rabbits the males were produced in large numbers, even exceeding the females, whereas the production of filariform larvae was strikingly reduced. Similar results were observed by the writer in cross-infection experiments on rabbits with the sheep strain of *Strongyloides*.

Sandground (13) recorded peculiar results through establishing *S. fülleborni* and *S. stercoralis* in abnormal hosts; the former, which normally showed only the pure indirect type of development, on inoculation into dogs and man produced predominantly the direct type. Observations of a similar nature were recorded by Faust and Kagy (6), who used human and non-human primate strains of *Strongyloides*. They transferrred these strains to human volunteers, a macaque monkey, and a number of dogs, and their

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Assistant.*

results further demonstrated the instability of the free-living phase in hosts other than normal.

Accordingly, infection with *S. agoutii* was attempted in the guinea pig, which was selected for several reasons. Firstly, the guinea pig is a member of the same family as the agouti, and for that reason it was hoped that the presence of *S. agoutii* would be tolerated by this host. Secondly, the guinea pig does not appear to be readily infected with most helminth parasites and had been but little used as an experimental animal in helminthological research. As far as the writer is aware, there are only three recent references to infection of the guinea pig with *Strongyloides:* Krediet records an undetermined species from it; Brumpt (3) transferred *S. papillosus* from sheep to guinea pigs; and Sheldon and Otto (17) infected them with *S. ratti*, while testing the host specificity of this parasite.

Materials and Methods

The source of supply of *Strongyloides* material for this work was the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The housing, feeding, and handling of these rodents were described in a previous paper (10), as were the methods of making and examining the routine faecal cultures.

As a general rule, experimental animals were infected percutaneously with filariform larvae of *S. agoutii*, usually obtained from the lid of a Petri dish to which they migrated readily from the faecal mass. If not so readily available, the Baermann isolation technique was adopted with modifications. The majority of the larvae were isolated within an hour, provided the culture was not unduly thick and massive; by careful disposition of the culture, larvae and other free-living forms may be drawn off comparatively free of debris. This procedure would not be applicable to large masses, but is well adapted to small faecal cultures containing large numbers of larvae. *Strongyloides* larvae become quickly inactivated if stored in deep water. Therefore, it is preferable to draw them off frequently into Syracuse watch glasses and concentrate immediately prior to use for infection purposes.

A modification of this technique was also adopted as a confirmatory measure in some post-mortem examinations. On certain occasions it was desired to determine whether larvae of immature adults were present in the lungs, heart, and portions of small intestine.

As a general rule an anaesthetic was given when larvae were administered percutaneously. For complete anaesthesia, Nembutal was injected intraperitoneally at the rate of 1 cc. per 5 lb. of live weight; anaesthesia was complete in a few minutes, and lasted from 1 to 3 hr. A greater measure of success was observed by percutaneous than by oral administration of larvae. The guinea pigs were approximately eight weeks old unless required older for some specific experiment. A small area of about one square inch was shaved on the animal's abdomen, rinsed with water to remove soap, then with alcohol,

and allowed to dry. The infective larvae were applied to the spot by means of a pipette, and the area was supplied with larvae and moisture for 15 to 20 min. It was then permitted to dry and the guinea pig kept under observation until again active. On no occasion did this method fail to establish a positive infection when suitable host animals were employed.

The eggs of *S. agoutii* are in the late stages of segmentation or embryonated when evacuated in the faeces. The routine faecal examination was used for diagnostic purposes only: quantitative output of ova was not considered necessary for the interpretation of results. A sugar centrifugal-flotation technique was used, and about 1 cc. of faeces was taken for examination.

For collection of faeces the animals were kept individually in wooden cages with ¼ in. wire mesh bottoms. The faeces dropped through onto paper or into water, as required. At times it was desirable to collect the total output for several days. A four-sided funnel of sheet zinc was attached to the bottom of the cage by hooks; the seams of the zinc were soldered so that daily washing would remove dirt or urine sediment. A beaker was placed beneath to collect the pellets.

All infected animals were kept in metal cages with wire floors, and no contamination or re-infection of an individual was experienced throughout these studies. The guinea pigs were identified by their colour markings, as only small numbers were used at a time. Tattoo marks were used in the ears of rabbits and the ears and tails of rats and mice were cut for identification.

Prior to post-mortem examination, the animals were starved for at least 12 hr. The entire small intestine was removed by cutting at the pyloric end of the stomach and at the caecum. The duodenum was then separated and the remaining length of intestine divided into two approximately equal parts. Since the number of individuals obtained at autopsy was not significant in any of these experiments, a method of collecting and fixing the adults en masse was adopted. The portion of intestine was slit open with a sharp scissor blade and covered with normal saline in a small Erlenmeyer flask. After half an hour the flask was stoppered and shaken gently. This procedure was repeated two or three times and the intestine removed. Most of the worms were detached by this time and found among the floating mucus. The contents of the flask were poured slowly into a very fine tea strainer, which held back most of the nematodes, or into a strainer covered with bolting cloth, which retained all.

The worms were carefully washed off by a fine jet of water into a petri dish, in which they were preserved. The majority of the worms parasitized the upper half of the small intestine of the guinea pig, the greatest concentration being found in the duodenum and the first few inches of the jejunum. Adults were obtained frequently from the ileum but only on rare occasions from the caecum or colon.

All animals used (with the exception of the agouti) were born and raised at this Institute under conditions that precluded any possibility of previous infection with *Strongyloides* species.

The Infection of an Experimental Host with *S. agoutii*

The tolerance of the guinea pig to *S. agoutii* and its desirable qualities as a laboratory animal provided ample opportunity for investigation.

Experiments were outlined to yield data on the minimum time required for parasitic females to reach maturity, to note the type of free-living development that would occur after the passage of the strain through an experimental host, and to record the length of life of the infective larvae.

After primary infection with the agouti strain, infections were transferred serially from one guinea pig to the next. This procedure excluded the possible introduction of a new strain into the series and any change occurring in mode of development could thus be attributed to some change in type. Owing to the peculiar results recorded, it was considered desirable to repeat the serial infection of guinea pigs, and two analogous series were started.

By preliminary trial, the prepatent period of the parasitic females was found to be approximately eight days. Faeces samples were examined from the sixth day following infection. Faecal tests were continued daily until the individual showed a heavy output of ova, whereupon examinations were made weekly until the test was negative for two or three weeks, when examination was discontinued. The exact date that ova ceased to be passed was not recorded. On several occasions experimental animals appeared to become constipated at the time the female parasite commenced ova production. This unavoidable condition extended the prepatent period for one day or longer, in some instances.

As soon as large numbers of ova were detected, three samples per individual were collected on different days for culturing to observe the mode of development.

Because the ova are in the late stages of segmentation when expelled, it was not considered necessary to examine cultures for 20 to 24 hr. Examination of cultures was concluded as soon as the activity of the filariform larvae ceased. It will be noted that the total number of cultures recorded graphically does not always correspond exactly with the total number of cultures examined. These omissions were unavoidable on account of sickness and holidays which occurred during the investigation. Some animals employed in these studies died as a result of over-infection, some were killed to obtain specimens of the parasitic generation of *S. agoutii*, and others were held for future use in immunity studies.

Serial Infection of the Guinea Pig. Series I

In this series, serial transfer of the agouti strain of *Strongyloides* was made through 27 guinea pigs. The experiment extended from April 1936 to November 1937, and during that period 92 cultures were made and 1,314 observations recorded.

Filariform larvae used for infective purposes ranged from 4 to 17 days in age, though the majority were from 4 to 6 days old. All the animals were

young males except guinea pig No. 21, a three-months-old female. Cultures from No. 24 were not examined in detail, but filariform larvae were obtained for transfer purposes. No. 27 died suddenly, terminating this series.

Transfer of *S. agoutii* through the guinea pig resulted in a marked change in the mode of free-living development. In the agouti, this strain showed an indirect or heterogonic mode of development, whereas in the guinea pig a mixed type was observed in the first 19 guinea pigs. The term "mixed" is used to indicate that the development observed was not entirely direct, for in addition to the production of filariform larvae, free-living males made their appearance. Only occasionally were no males found.

This mode of development did not persist throughout the entire series, since cultures from No. 20 showed the presence of a few free females in addition to the filariform larvae and free males, a modified reversion to the indirect type of the agouti. Cultures from No. 21 continued to show the presence of free females in quite large numbers, though cultures from Nos. 22, 23, 25, and 26 produced only filariform larvae and free males.

This mixed type of development presented an excellent opportunity for recording the period of activity of free males, the first appearance of filariform larvae of direct development, and the longevity of these larvae, in faecal cultures. Such data are recorded in Figs. 1–4. Free males occurred in the majority of cultures on the second and third days, but only once on the first day; they did not appear after the fourth day (Fig. 1). In the greater number of cultures the males became inactive on the fourth and fifth days, though in one instance they were active on the seventh day. In only one instance were males observed in cultures for more than four days, the longevity in most cases being from two to three days. Filariform larvae occurred in the majority of cultures on the third day, though many were recorded on the second and fourth days; in a few instances they did not appear until the fifth day (Fig. 2). The duration of life of filariform larvae in cultures was easily recorded with this mixed type of development.

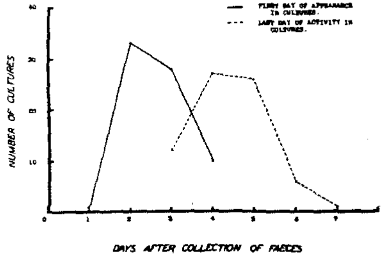

FIG. 1. *Frequency of first day of appearance and last day of activity of free-living males of S. agoutii in faecal cultures from the guinea pig.*

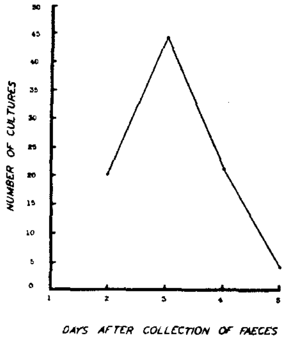

FIG. 2. *Appearance of filariform larvae of S. agoutii in cultures after passage of faeces by guinea pigs.*

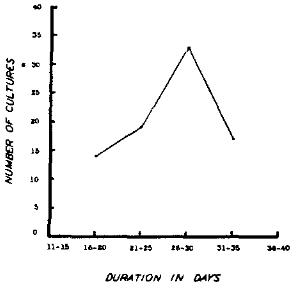

FIG. 3. *Longevity of filariform larvae of S. agoutii in faecal cultures from the guinea pig.*

Fig. 3 indicates that the greater proportion of cultures died between the 26th and 30th days, while no cultures became inactive prior to 16 days, or continued to show active larvae after 35 days. The appearance of filariform larvae on the third day in the greater number of cultures will permit the deduction that the maximum life of larvae is 32 days. No evidence of lack of viability of larvae was observed as the result of serial transfer.

Of the 27 guinea pigs in this series, 21 were killed and given a thorough post-mortem examination, one was killed accidentally and received no autopsy, and five were used for other investigations. Nine of the 21 were killed to obtain adult *Strongyloides*, while the other 12 were killed some months after infection in order to check the negative faecal tests that were being recorded.

None of these 12 animals revealed any stages of *Strongyloides* in the lungs, heart, liver, small or large intestine. It is therefore considered that the infection of these animals was eliminated rather than that the parasitic females were becoming non-fecund.

Serial Infection of the Guinea Pig. Series II and III

Owing to the peculiarities observed in the free-living phase of Series I, two further series were commenced to repeat the observations under conditions as nearly identical as possible. The data obtained relating to longevity of free forms and length of life of cultures displayed such similarity to the results shown graphically for Series I that they did not seem of sufficient importance to warrant repetition.

Series II extended from July 1937 to June 1938, and during this period *S. agoutii* was serially transferred through 15 guinea pigs, 92 faecal cultures were prepared, and 919 observations were recorded. Filariform larvae used for infection purposes ranged from 3 to 18 days in age; in most cases, four-day-old larvae were used. Guinea pigs Nos. 1 and 2 were the only females in this series.

The mode of development was very inconsistent. Cultures from Nos. 1 and 3 yielded a few free females in addition to the filariform larvae and free males. Cultures from No. 7, however, gave filariform larvae and free females almost exclusively and in large numbers, though one or two males were observed. All other cultures produced filariform larvae and free males only; in a few cases, no males were observed. Since no constancy in mode of development was apparent, the series was discontinued after the fifteenth individual was infected. Of the 15 guinea pigs, six were killed by over-infection or to obtain adult parasites, six were killed after some long period to check reliability of faecal examinations, and three were used for further studies.

The studies in Series III consisted of the serial transfer of *S. agoutii* through 12 guinea pigs, from January to July, 1938, and included 60 cultures and 804 observations. The larvae used for infection purposes ranged from 3 to 12 days old, but were usually four days old.

The type of development in Series III was also variable. A few free females were recorded from cultures from guinea pigs Nos. 3 and 10. The remaining cultures tended to show direct development, with free males recorded intermittently throughout. Because of the variable results, the series was concluded after infection of No. 12. Eight of the 12 animals were killed or died as a result of over-infection. Adults were obtained from five only, the other three being negative. The remaining four animals were used for further study.

The Free-living Development of *S. agoutii* from the Agouti and Guinea Pig

In a previous paper by the author (10) on *S. agoutii* of the agouti, the indirect mode of development of the free-living generation was found to prevail. In this study, it has been shown that a mixed type appears in cultures of

faeces from guinea pigs infected with *S. agoutii*. This mixed type is represented by filariform larvae and free-living males, though free females have appeared. Two types of free-living development have thus been shown for this strain of *Strongyloides* in different hosts.

An attempt was made to ascertain whether the factors influencing the type of development could be attributed to the host or to the environment of the culture media. A young male guinea pig was infected percutaneously with *S. agoutii* from the agouti. Upon appearance of ova in its faeces, three groups of cultures were made: (i) faeces; (ii) ova removed from faeces by crushing, sieving, washing, and sedimentation, were seeded on guinea pig faeces from uninfected young stock and (iii) on sterile agouti faeces (autoclaved at 14 lb. pressure for 15 min.). Sterility of this agouti faeces, and also of faeces from the uninfected guinea pigs, was verified by control cultures.

To obviate error in culture examination, these were examined daily under the binocular microscope, and a sample was examined by the Baermann technique. When cultures yielded only filariform larvea or no larvae for five or six days, examinations were discontinued.

Of 10 cultures in group (i), all showed mixed development, as would be expected. In group (ii), 10 cultures all yielded filariform larvae and free males, with one free female in one culture. In group (iii), three out of four cultures showed the indirect mode of development, with one larva observed on the first day in one culture. A further 13 cultures in this group showed seven with indirect development, five with no development, and one with two larvae on the fourth day.

In the second and third groups, the population of the cultures was low in many cases. The uncontrolled process employed in seeding cultures with ova, which was not carried out quantitatively, may possibly explain this.

Discussion of Free-living Development of Strongyloides

In many instances when a species of *Strongyloides* is experimentally established in a new host of different taxonomic position from its usual or normal host, the life cycle is observed to be radically changed from the type manifested in the original host. Inadequate observations have on occasion led to the belief that a certain strain is exclusively direct or indirect, whereas actually few such strains appear to exist.

From the data obtained on the free-living phase of cultures from Series I, definite peculiarities in the mode of development were observed. By transfer of the agouti strain of *Strongyloides* through the guinea pig, a direct type of development might be expected. In these studies a modified direct mode was recorded, being represented by filariform larvae and free males. This mixed development was evidenced in cultures from the first 19 animals of the series, after which free females were recorded. Similar observations were recorded in Series II and III, though females occurred with considerable irregularity and inconsistency.

In an attempt to explain this phenomenon the current hypotheses will be reviewed as briefly as possible. Sandground (13) regards the parasitic female as syngonic, giving rise to ova which develop to males or females. If ova are fertilized in equal numbers, theoretically both sexes would be reproduced in equal numbers, but this seldom occurs. If only females or males are produced in the bi-sexual generation, he considers that the type of sperm that determines the mixing sex has either not been produced or has degenerated without functioning. Direct development might readily be attributed to parthenogenesis, but Sandground found the same syngonic condition occurred in parasites that were known to have been producing the direct type of development almost exclusively. However, the finding of sperm in his syngonic females does not completely exclude the occurrence of parthogenesis. Sandground further provides an explanation for direct development by consideration of the occurrence of an atypical distribution of the chromosomes at mitosis, which would constitute the determining mechanism for this mode. He suggests auto-infection as an explanation for direct development, on the hypothesis that in an infection originally showing both direct and indirect modes, the larvae of direct metamorphosis may, by auto-infection, reinforce the existing infection. Since larvae have been shown to develop mainly according to the parental history, it will follow that with the increasing age of an infection, the direct mode will finally predominate. Sandground (13) has observed in daily cultures of material from a rat, that within a short period, the sex-ratio may range from a bisexual generation almost entirely of females to another of males only. Following the revolutionary discovery of the parasitic male by Kries (12) and Faust (5), the latter proposed a possible explanation for alternative modes of development. He suggested that fertilized ova may give rise to an indirect mode of development, unfertilized to a direct mode. After the original supply of spermatozoa stored in the females becomes exhausted, the progeny of parasitic females (by parthenogenesis) in the intestine would be direct only, since the parasitic males are usually found in the bronchioles and consequently would not re-fertilize the females.

Following his investigation with *S. simiae*, Beach (1, 2) proposed the theory that under optimum conditions indirect development consistently occurred, whereas unfavourable conditions tended to modify the strain toward a direct type. Beach considers that the sex is established before or at the time of formation of the egg, but the development is modified by factors such as nutrition, viscosity, and toxicity. He further suggests that filariform larvae, appearing as a result of changing from an indirect to a mixed type are, in reality, potential sexual individuals. His work strongly indicates that directness and indirectness are conditioned by environment and not by genetic constitution. Graham (9), however, presents evidence indicating that the mode of larval development followed by *S. ratti* is determined prior to oviposition.

The occurrence of parthenogenesis in the first 19 experimental animals of Series I could readily explain direct development if no free males had been present. Chance hyperinfection of guinea pig No. 20 might have permitted fertilization of a parasitic female with subsequent production of indirect development. However, since the ova of *Strongyloides* are evacuated from the guinea pig in the late stages of segmentation (although usually embryonated in the agouti), it seems improbable that Faust's hyperinfection or Sandground's autoinfection would occur.

Parasitic males were not observed on any occasion, which supports the suggestion of Faust that unfertilized ova may produce a direct type of progeny. However, this fails to account for the females in the twentieth generation, and is inadequate to explain the free males throughout the series.

The conditions experienced by the *Strongyloides* strain in cultures of guinea pig faeces may have been sufficiently unfavourable to influence the strain to a direct mode of development. But as all guinea pigs received materially the same rations and all cultures were made under as nearly identical conditions as possible, this influence would be constant throughout the series.

Beach (2) considers that filariform larvae, produced when first-generation rhabditiform larvae are grown on artificial media, are potential free-living adults. He has demonstrated the alteration of a supposed indirect type of development of *S. simiae* to a mixed type when the worms were grown on artificial media. This mixed type showed large numbers of first-generation filariform larvae, a considerable number of males, and few or no females. On addition of small amounts of aqueous extracts of monkey faeces to the media, a more favourable growth was induced, and the mixed development tended to revert to the original mode, i.e., there was an increase in females and a decrease in the filariform larvae. These observations appear comparable with the data of the present study, and Beach's deductions are more readily applicable than any previously discussed. It seems logical to assume that indirect development is normal for *Strongyloides*, direct development occurring when unsuitable conditions are encountered. As a result of this adaptation, filariform larvae of the direct mode are considered as suppressed free females and males, which manifest their true forms only in the presence of certain definite requirements.

In these studies, the composition of guinea pig faeces apparently does not completely furnish the necessary medium for the appearance of free females and males with regularity, though males have appeared more consistently than females.

The results obtained from the culture of ova of *S. agoutii* from the guinea pig on sterile agouti and guinea pig faeces present strong evidence in support of the theory that directness and indirectness are conditioned by environment. These two cultures of ova displayed different modes of free-living development. The former reverted to the original indirect type of the agouti strain; the latter showed the development of free males and filariform larvae, as usually observed in cultures from a guinea pig infected with *S. agoutii*.

A number of cultures of agouti faeces seeded with ova from guinea pig faeces showed no development. This condition may probably be attributed to the uncontrolled method of distribution of ova on cultures or to some unknown factor preventing development of the ova.

The mechanical methods employed in obtaining these ova did not affect the mode of development of larvae. From the results obtained it is assumed that the factor controlling the mode of free-living development of *S. agoutii* is directly associated either physically or chemically with the media in which the eggs are cultured.

The Prepatent and Patent Periods of *Strongyloides agoutii* in the Guinea Pig

The prepatent and patent periods in members of the genus *Strongyloides* have been variously determined and have shown considerable diversity for different species and strains. Sandground (14) reported the prepatent period of *S. stercoralis* to be 5 to 10 days in dogs and 9 to 16 in cats. Faust *et al.* (7) recorded infections of dogs with human strains of *S. stercoralis* in which the prepatent period usually ranged from 11 to 18 days. Graham (8), using single larva infection of rats with *S. ratti*, showed the prepatent period to range from 5 to 11 days, most individuals being positive by culture on the sixth day, whereas Sheldon (15) recorded the prepatent period of *S. ratti* as ranging from 3 to 6 days.

In these studies, the three series of guinea pigs infested with the agouti strain of *Strongyloides*, together with a few other individuals employed in immunity investigations, gave considerable data on the period between infection and the appearance of ova in the faeces. All animals were tested on the sixth day after infection and daily thereafter. The frequency distribution of the first day of appearance of ova, after exposure to filariform larvae, is recorded in Fig. 4. In spite of the many factors that may impede the migration of larvae to the intestine or delay the discharge of eggs, it is of interest to note the narrow range to which the first appearance of ova is confined. Of the 58 animals, 24% were positive by faecal test on the seventh day, 47% on the eighth, 22% on the ninth, and 7% on the tenth day; that is, 71% were positive by the eighth day, the modal point for the series.

These observations show the commencement of the patent period to be confined to comparatively narrow limits; in fact, these limits are considerably narrower than those at the end of this period. The patent periods reported for the genus *Strongyloides* are found to show considerable variation. Sandground (14) has found the duration of *S. stercoralis* infection to be from 2 to 11 months in dogs and from 2 to 7 months in cats. Faust *et al.* (7) state that infection of the dog with human strains of *Strongyloides* is maintained for only a period of weeks or months, as contrasted with a period of years in man, the natural host. The patent period of *S. ratti* has been shown by Sheldon (15) to range from 51 to 136 days. In the present studies with *Strongyloides*, the period over which eggs have been passed in the faeces has been found to show

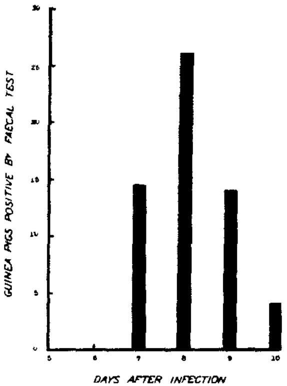

FIG. 4. *Frequency distribution of first day of appearance of ova in faeces of 58 guinea pigs after infection with S. agoutii.*

considerable variation. Eggs were recorded in the faeces of 26 guinea pigs for three to eight weeks. The majority of infections terminated during the fourth or fifth week. A number of individuals were killed subsequent to a negative faecal test, and autopsy showed that the test was a true indication that the infection had disappeared rather than that the females become non-fecund. As the animals were infected with filariform larvae en masse, no data were available to show if the heavier infections were lost more quickly than the lighter ones. It was observed, however, that the older the host at the time of infection, the earlier the infection was lost.

Observations on Migration of Larvae of *S. agoutii* Through the Guinea Pig

It is generally agreed that, following percutaneous infection, the path of migration of *Strongyloides* necessitates the passage of the larvae through the lungs prior to their establishment in the small intestine. To ascertain the rate of migration and to observe the organs in which the various stages were found, four guinea pigs were infected and killed at intervals over a period of eight days.

The animals employed were young males, six weeks old at time of exposure. They were infected en masse with *S. agoutii*, as previously described. Autopsies were made and all suitable organs examined by the Baermann technique to obtain any migrating larvae. In each case, the autopsy included the examination of the trachea, lungs, liver, heart, spleen, kidneys, stomach, small and large intestines.

The first guinea pig was killed 48 hr. after exposure and one filariform larva was obtained in the heart. The second was killed after 72 hr.; a few larvae were observed from the lungs, heart, stomach, duodenum, and jejunum. The third was killed after 96 hr. and yielded filariform larvae from the heart and stomach: larvae in stages of metamorphosis between the filariform larva and the adult were found in the duodenum and jejunum. The fourth was killed on the eighth day, and one filariform larva was obtained from the heart; the duodenum and jejunum yielded large number of both immature and ovigerous females, and a few were also found in the ileum.

As a result of these infections, it appears that with this species the migration through the lungs is of a transitory nature; no forms other than filariform larvae were observed in that organ. The time of migration through the blood stream extends over a considerable period of time, since a larva was collected from the heart as late as the eighth day subsequent to infection.

Acquired and Age Resistance of the Guinea Pig to Infection with *S. agoutii*

The subject of resistance and immunity of the host to infection with helminths has received but little attention until recent years. From the point of view of the host there are three major types of resistance mechanism. The first type is natural immunity, which is found frequently among animals. Most individuals are found to be naturally immune to parasites of unrelated animals, though wide adaptations are known to exist. The second type is that of age resistance. This is displayed by animals that may be susceptible in early life to infection with a parasite, but become less susceptible later. The third type is that which is acquired by the host as a direct consequence of infection with a specific parasite.

Previous work on resistance to re-infection among members of the genus *Strongyloidès* is scanty, and Sandground (14) appears to have been the first to make studies of this nature. He reported investigations on susceptibility, resistance, and acquired immunity of dogs and cats to infection with *S. stercoralis*. Successful infection of dogs and cats of all ages was brought about with a human strain of *S. stercoralis*, and animals that lost their infection were found to be refractory to re-infection; the acquired immunity in dogs lasted for more than six months. It was concluded that in immune animals the larvae reach the intestine but do not attain sexual maturity. Kotlan and Vajda (11) report an age immunity to *S. ransomi* occurs in pigs. Sheldon (16) carried out quantitative studies on acquired resistance and showed that a marked resistance was acquired by rats as the result of super-infection with *S. ratti*. He also demonstrated that resistance was acquired and retained by rats as the result of a single previous infection that had run its course and disappeared; they were also found to be successfully immunized by serial injections of heat-killed larvae in a saline suspension. Animals two or four months old were found to be equally susceptible to infection with *S. ratti*;

at eight months resistance was observed, at 12 months was less apparent, and was observed again at 17 months.

A series of experiments was undertaken to determine if an acquired or an age resistance was manifested by guinea pigs to *S. agoutii*. If an acquired resistance was exhibited, it was hoped to discover whether this tended to pass off after a time or if it inhibited reproduction of the female or prevented the parasite from establishing itself in the intestine with the resultant expulsion of the worm in the faeces.

A total of 17 guinea pigs was used. Several of these had been used in the experiments on serial transfer, thus providing excellent material on which to attempt re-infection with the same parasite. Infection of the guinea pigs was effected percutaneously, as described previously. Diagnosis of infection was determined by faecal test.

The age of animals employed in these tests varied to a considerable extent. Control guinea pigs were employed to check the infectivity of larvae used for re-infection trials. Post-mortem examinations were not made on all guinea pigs as some were used for other purposes. In certain instances when negative faecal tests were recorded, autopsy was carried out to ascertain if females were present but had been rendered non-fecund by an immunity mechanism. In no case was this found to occur.

The essential data relating to this series of infections are presented in the accompanying tables. To determine if any resistance existed, a preliminary trial was initiated, the results of which are presented in Table I. Three guinea pigs had previously been infected with *Strongyloides*. Prior to the second exposure they were shown by faecal test to have eliminated their primary infection. This exposure was about six months after the first; they did not become re-infected. A third exposure was made one month after the second but no evidence of infection was observed. No autopsy was carried out.

The second group comprised four individuals previously infected and two controls (Table II). These four animals gave a negative faecal test for several months before re-infection was attempted. Since guinea pig No. 4 was exposed to larvae from a different culture to that used as a source of larvae for Nos. 5, 6 and 7, two young controls were exposed to ensure the infectivity of both lots of larvae. The guinea pigs were all exposed to infection on the same day, and subsequent faecal tests yielded negative results for Nos. 4–7; both controls were positive. Chandler (4) has shown that a resistance acquired as a result of a previous infection of *Nippostrongylus muris* in rats may materially interfere with reproduction of the parasite and cause a pronounced reduction in egg output. In view of this fact it was considered advisable to autopsy Nos. 4–7, to make sure that the routine faecal tests were not yielding inaccurate results. Nos. 5 and 6 were autopsied 14 and 13 days after infection, Nos. 4 and 7, 31 days after infection. Thorough examination of the lungs, heart, liver, small and large intestines did not yield any stages of *Strongyloides*. The control to No. 4 was killed and examined on the 23rd

TABLE I

PRELIMINARY TRIAL OF EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., *months*	Date of 1st expos., 1937	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure		Date of 3rd expos., 1938	Faecal tests after *3rd exposure*	
			Date	Result		Date	Result		Date	Result
1	4½	June 17	June 25, 26, 28, 29 July 15 Aug. 16 Oct. 13, 27 Dec. 1, 21 Jan. 1	Positive Positive Positive Negative Negative Negative	Jan. 27	Feb. 3–5, 9–11, 15, 22, 25, 28	Negative	Mar. 2	Mar. 9–12, 14, 15, 22	Negative
2	5	July 15	July 24, 26, 27 Aug. 5, 16 Oct. 10, 26 Dec. 1, 21 Jan. 12	Positive Positive Negative Negative Negative	Jan. 27	Feb. 3–5, 9–11, 15, 17, 19, 22, 25	Negative	Mar. 2	Mar. 9–12, 14, 15, 22	Negative
3	2	July 27	Aug. 5, 6, 16 Oct. 13, 19, 27 Dec. 12 Jan. 1	Positive Negative Negative Negative	Feb. 2	Feb. 9, 10, 15, 17, 20, 22, 25, 28	Negative	Mar. 2	Mar. 10–12, 14, 15, 24	Negative

TABLE II

EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos., 1938	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure	
			Date	Results		Date	Results
4	12	April 1	April 9–11, 13, 19, 27 May 16, June 6, 20 July 18, Aug. 9. Oct. 13, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12–17, 19, 20, 27	Negative
Control	1½	Dec. 5	Dec. 12–17, 19, 20	Positive			
5	2	Jan. 10	Jan. 19, 20, Feb. 3, 17 Feb. 22, 28, Mar. 22 April 4, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 13, 17, 24	Positive Negative Negative Negative Negative	Dec. 5	Dec. 12, 14, 15, 17, 18	Negative
6	2	Mar. 10	Mar. 17, 18, April 5 April 19, May 5 June 6, 20, July 18 Aug. 8, Oct. 13, 17, 20	Positive Negative Negative Negative	Dec. 5	Dec. 12–16	Negative
7	2	Mar. 29	April 4, 6, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 10, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12–20, 27	Negative
Control	2	Dec. 5	Dec. 14–17, 19	*Positive*			

POST-MORTEM EXAMINATION

No. 4. Killed Jan. 5, 1938. All organs (lungs, heart, liver, duodenum, jejunum, ileum, large intestine) negative for *Strongyloides*.
Control. Killed Dec. 28, 1938. *Small intestine heavily infected with adult Strongyloides.*
No. 5. Killed Dec. 19, 1938. All organs negative for *Strongyloides*.
No. 6. Killed Dec. 18, 1938. All organs negative for *Strongyloides*.
No. 7. Killed Jan. 5, 1939. All organs negative for *Strongyloides*.

day and displayed a heavy infection of *Strongyloides* in the small intestine; the other control guinea pig was used for further experimentation.

To check these results another experiment was undertaken in which three previously infected, and two control, animals were employed. The essential data on this trial was summarized in Table III. Before attempting infection for the second time, the guinea pigs were shown to have been negative to faecal tests over a period of some months. One control was employed to test the infectivity of larvae used for application to Nos. 8 and 9, another for No. 10. After attempting infection, these animals received the usual routine faecal examinations, negative results being recorded in all cases. Nos. 8 and 9 were autopsied on the 9th and 11th days after exposure; the control was killed on the 8th day. No autopsy of No. 10 and the control was carried out since they were held for further use. Post-mortem examination of Nos. 8 and 9 did not reveal any stages of *Strongyloides* in the intestine, whereas one larva was recorded from the heart of the control, and the small intestine was heavily infected with adults.

TABLE III

EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos.	Faecal tests after 1st exposure		Date of 2nd expos., 1939	(F) Faecal and (B) Baermann* tests after 2nd exposure	
			Date	Results		Date	Results
8	2	Dec. 20 1937	Dec. 30, Jan. 17 Feb. 3, 20, 22, Mar. 15, 22 April 5, 19, May 16 June 6, 20, July 18 Oct. 13, 17, 24	Positive Negative Negative Negative Negative	Jan. 9	(F) Jan. 16–18 (B) Jan. 10–18	Negative Negative
9	2	May 13 1938	May 20–22, June 18, 20 Aug. 8, Oct. 13, 17, 25	Positive Negative	Jan. 9	(F) Jan. 16–20 (B) Jan. 10–20	Negative Negative
Control	1½	Jan. 9 1939	Jan 16	Positive	Jan. 9		
10	2	June 2 1938	June 10, 13, 20, July 18 Aug. 9, Oct. 13, 17, 25	Positive Negative	Jan. 30	(F) Feb. 7, 9–11, 13–15, 20, 23 (B) Jan. 31–Feb. 8	Negative Negative
Control	1½	Jan. 30 1939	Feb. 7–9, 10, 13, 20, 27 Mar. 21	Positive Negative			

* *Baermann examination was made in morning and late afternoon each day.*

POST-MORTEM EXAMINATION

No. 8.	Killed Jan. 18, 1939.	All organs negative for *Strongyloides*.
No. 9.	Killed Jan. 20, 1939.	All organs negative for *Strongyloides*.
Control.	Killed Jan. 17, 1939.	1 larva in heart. Intestine heavily infected with adult *Strongyloides*.

From Table III it will be seen that for the first 8 to 10 days following the second exposure to infective larva, a Baermann examination was introduced in addition to the routine faecal test. When an acquired resistance is manifested by an individual, there appears to be a generalized condition which may inhibit development to the adult stage or prevent successful establishment of the invading parasite within the intestine. In view of this fact and on the assumption that the migrating infective larvae would, if they arrived at the intestine, still be active, collection of the total faecal output of Nos. 8, 9, and 10 was made twice daily and Baermanned, the sediment being drawn off after one-half and one hour. It was anticipated that if the migrating larvae did not become established in the intestine they would be passed out, but no larvae were found.

Since a number of the individuals employed in the preceding trials were, on an average, about one year old at time of re-exposure, an attempt was made to determine whether this resistance was due to an age factor. Three guinea pigs known to be considerably more than a year old were used for this purpose, the data on which are recorded in Table IV. All three were

TABLE IV

Infection of adult guinea pigs with infective larvae of *Strongyloides agoutii*

Guinea pig	Date exposed, 1938	Faecal tests	
		Date	Results
A	Dec. 29	Jan. 7–10, 12, 16, 18, 20, 23, 25, 30 Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
B	Dec. 29	Jan. 7–10, 12, 16, 20, 23, 25 Jan. 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
C	Dec. 29	Jan. 7–10, 12, 16, 18, 20, 23 Jan. 25, 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative

infected on the same day and faecal tests throughout the month following exposure were all positive. The infection was observed to pass off after the first month; these animals were not autopsied but were held for further use.

The preceding experiments show that the guinea pig acquires a resistance to re-infection with *Strongyloides*. This resistance does not permit the establishment of the parasite in the intestine, and animals that have lost their infections cannot be re-infected over a period ranging at least from 6 to 13 months after the primary infection. An attempt was made to ascertain whether infective larvae were expelled from the intestine with the faecal material; results of observations were negative but because of complicating factors these observations are not considered conclusive.

Acknowledgments

It is with very much pleasure that the author expresses his sincere appreciation and thanks to Professor T. W. M. Cameron for his guidance and generous assistance in the direction of these studies.

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THE HELMINTH PARASITES OF SLEDGE-DOGS IN NORTHERN CANADA AND NEWFOUNDLAND[1]

By Thomas W. M. Cameron[2], I. W. Parnell[3], and L. L. Lyster[4]

Abstract

In a survey, based on examination of faeces and viscera of sledge dogs, the following were identified: hookworms, ascarids, whipworms, kidney worms, fish-carried and other tapeworms, the Canadian liver-fluke and other trematodes and an acanthocephalid. The distribution of the infections is noted. The acanthocephalid, *Corynosoma semerme*, is recorded for the first time from North America.

Since 1933, the Institute of Parasitology has been engaged in a survey of the parasites of animals in Canada. During that period it has accumulated a considerable amount of data on infections of economically important animals in the Arctic and sub-Arctic regions.

The parasitic fauna of animals is of considerable importance to the inhabitants of these regions. The aboriginal peoples live entirely on the animals that breed there and although of an advanced culture, many, particularly Esquimaux, eat a large part of their meat and fish uncooked. Because of the rigour of the climate, human beings and domestic animals live close together and make the transmission of helminths a simple matter. Because of the severe climate, bacterial infections are somewhat rare and animal parasites have become of even greater significance.

The animal of greatest importance is the dog. Transportation and communication of white man and native, alike, depend almost exclusively upon this animal. Consequently an investigation of the parasites of this animal has been a first consideration. The data at our command are now sufficient to record and give an adequate picture of the geographical distribution of its parasites in these regions.

The information was mostly obtained through co-operation of the R.C.M. Police, the National Parks Branch, and the Hudson's Bay Company in Canada, and of the Newfoundland Rangers in Labrador. In addition, both junior authors have personally collected in the eastern Arctic, one (I.W.P.) in 1933 in the north and west sides of the Quebec Peninsula, and the other (L.L.L.) in 1939 from that region and from Baffin, Ellesmere, Southampton and Somerset Islands, and the west coast of Hudson's Bay.

Much information has been obtained by examination of faeces sent to the Institute by interested persons, including the R.C.M. Police, Game Wardens,

[1] *Manuscript received April 4, 1940.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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[3] *Lecturer in Parasitology.*

[4] *Graduate assistant.*

Newfoundland Rangers, and others. In most cases this material was unpreserved, and, although sometimes several months elapsed before it was delivered in Montreal, virtually all arrived in good condition. On a few occasions it was formalized. Most faecal material was shipped in two ounce tin ointment boxes, and all was examined by simple, repeated concentration. These examinations and identifications were made by the senior author.

In addition, dog carcasses or entrails have been sent to the Institute or examined on the spot. Those sent in (many coming in the "Nascopie") were formalized and shipped in a semi-mumified condition (e.g., superficially dry) in garbage cans. These cans were packed with moist material to prevent undue drying and in most cases the technique functioned adequately.

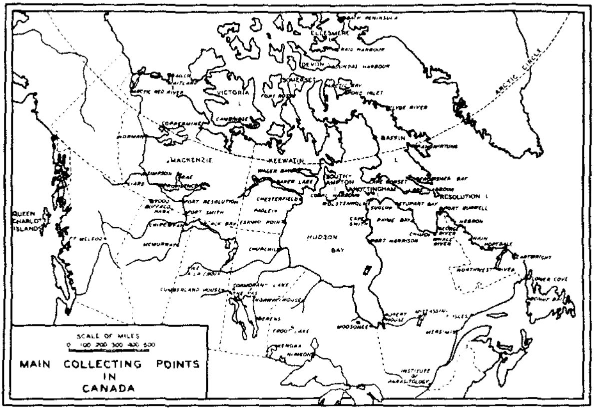

FIG. 1

In no instance was a parasite-free collecting point recorded although certain parasites have quite definite geographical ranges. The distribution is considered by species.

Nematodes

Hookworms

Hookworms have been found in dogs in all areas surveyed and they occur in the most northerly station in North America, viz., Craig Harbour, Ellesmere Island, N.W.T. In many cases, the diagnosis has been based only on eggs. As, however, only the northern dog hookworm (*Uncinaria stenocephala*) has been found (on autopsy) north of Moosonee, it is probable that all these records refer to this species.

Egg records are as follows—

Arctic Red River, Mackenzie District, N.W.T.
Battle Harbour, Labrador.
Bonne Baie, Nfld.
Cambridge Bay, Victoria Island, N.W.T.
Cartwright, Labrador.
Chimo, Ungava Bay, Que.
Craig Harbour, Ellesmere Island, N.W.T.
Flower Cove, Nfld.
Fort Fitzgerald, Alta.
Fort George, Man.
Hebron, Labrador.
Hopedale, Labrador.
Lake Harbour, Baffin Island, N.W.T.
Maitland Point, Mackenzie District, N.W.T.
McLeod Lake, B.C.
Moosonee, James Bay, Ont.
Nain, Labrador.
Northwest River, Labrador.
Pangnirtung, Baffin Island, N.W.T.
Pine River, Man.
Port Burwell, Hudson Strait, N.W.T.
Port Harrison, Hudson Bay, Que.
Stupart Bay, Hudson Strait, Que.
William Harbour, Labrador.
Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, this species has been found in carcasses from Dundas, Moosonee, Burwell, Sept Isles, and Nipigon. Two dogs were positive at Dundas; of these one was born on Devon Island while the other had been imported from Pangnirtung on Baffin Island. This species also occurs in dogs on the Island of Montreal, P.Q., and is common in foxes throughout Eastern Canada.

Ancylostoma caninum, the southern dog hookworm, was found in one dog from Moosonee, in association with the northern species. This appears to be the most northerly record of its occurrence. It also occurs on the Island of Montreal, P.Q.

Ascarids

Ascarids are almost equally widely spread, the predominant species being *Toxascaris leonina* (syn. *T. felis*) although *Belascaris marginata* (syn. *Toxocara canis*) also occurs; these species were not separated in the writers' egg records, although most were *T. leonina*.

Egg records were obtained from:

Cartwright, Labrador.
Chimo, Ungava Bay, Que.

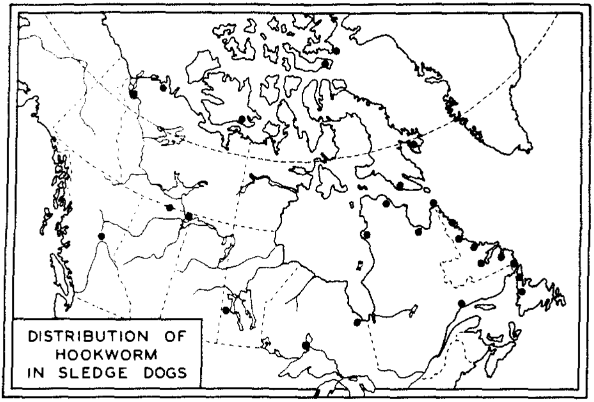

FIG. 2

Churchill, Man.
Cumberland House, Sask.
Eskimo Point, Keewatin District, N.W.T.
Fort Chipewayan, Alta.
Fort Resolution, Mackenzie District, N.W.T.
Hebron, Labrador.
Hopedale, Labrador.
Lake Harbour, Baffin Island, N.W.T.
Maitland Point, Mackenzie District, N.W.T.
Moosonee, James Bay, Ont.
Nain, Labrador.
Rupert House, James Bay, Que.
William Harbour, Labrador.
Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, actual specimens of *T. leonina* from dogs were collected from Cape Smith, Dorset, Moosonee, Nottingham, Stupart and Wolstenholme, *and of B. marginata from Moosonee and Wolstenholme.*

Whipworms

Whipworms (*Trichuris vulpis*) were seen on only one occasion (Kenora, Ont.) and were not recovered from Arctic regions at all. They are fairly common in southern Canada.

Kidney Worm

A single infection of *Dioctophyme renale* was recorded from Berens. In this case both kidneys were infected and the female worms reached a length of 30 in. The dog died as a result of this infection.

Tapeworms

Diphyllobothrium spp. At least two species of this genus appear to occur in dogs in the north of Canada. One, which is found in the Mackenzie River basin, is associated with fresh-water fish and is almost certainly *D. latum*. Most records were based on eggs in the faeces, but actual tapeworms found in man in northern Saskatchewan belong to this species, and the distribution is continuous from this region.

The other species, occurring in northern Quebec and along the Arctic coast, has a longer egg and the carrier fish may be assumed to be a salt-water one. The few specimens recovered from dogs in that region were in too poor a state of preservation for proper identification and could be referred neither to an existing species nor a new one. Accordingly, while the writers believe that at least two species exist, they have not attempted, at this stage, to identify them by name.

Eggs of tapeworms of this genus were found in the following places:

Arctic Bay, Baffin Island, N.W.T.
Baillie Island, N.W.T.
Berens, Man.

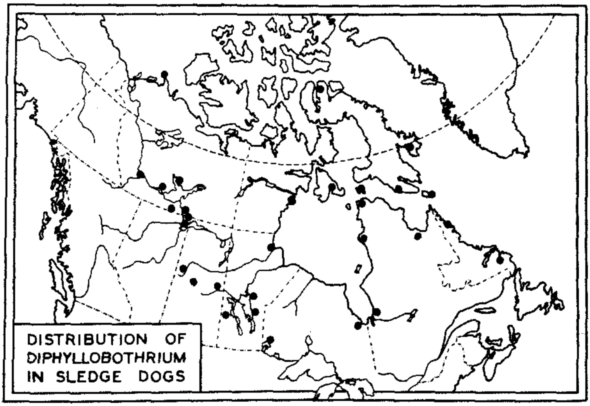

FIG. 3

Cameron Bay, Keewatin District, N.W.T.
Cartwright, Labrador.
Cat Lake, Ont.
Chesterfield, Keewatin District, N.W.T.
Chimo, Ungava Bay, Que.
Churchill, Man.
Coral Harbour, Southampton Island, N.W.T.
Cumberland House, Man.
Forteau, Labrador.
Fort Chipewayan, Alta.
Fort Fitzgerald, Alta.
Fort Providence, Mackenzie District, N.W.T.
Fort Smith, Mackenzie District, N.W.T.
Goldfields, Man.
Hebron, Labrador.
Ile a la Crosse, Sask.
Kenora, Ont.
Lake Harbour, Baffin Island, N.W.T.
Moosonee, James Bay, Ont.
Norway House, Man.
Pangnirtung, Baffin Island, N.W.T.
Pine River, Man.
Port Harrison, Hudson Bay, Que.
Rae, Mackenzie District, N.W.T.
Resolution, Hudson Strait, Que.
Rupert House, James Bay.
Simpson, Mackenzie District, N.W.T.
Waskesiu Lake, Sask.
William Harbour, Labrador
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens referable to this genus were found in Wolstenholme and Nottingham Island.

Taenia spp.

Five dogs showed *Taenia* eggs in their faeces, but again it was impossible to refer them to a species. These cases came from:

Cameron Bay, Keewatin, N.W.T.
Churchill, Man.
MacLeod Lake, B.C.
Pine River, Ont.
Rupert House, James Bay, P.Q.

Specimens of *Taenia*, not identifiable because of partial decomposition, were found in Notre Dame du Nord, Que., and Cape Smith.

Dipylidium caninum

This tapeworm was found in carcasses from Moosonee, Nottingham and Wolstenholme.

Trematodes

Metorchis conjunctus

The Canadian liver fluke was found in dogs from:

Berens, Man.
Cat Lake, Ont.
Cumberland House, Sask.
Ile a la Cross, Sask.
Kenora, Ont.
Lac la Ronge, Sask.
Lake Mistassini, Que.
Moosonee, James Bay, Ont.
Norway House, Man.
Pine River, Ont.
Rupert House, James Bay, Que.
Trout Lake, Ont.
Waskesiu Lake, Sask.

The southern limit of this parasite appears, from present records, to be the St. Lawrence and the Canadian border. Its northern limit in Quebec is unknown, but its western and northern limits appear to be the height of land in Saskatchewan and the borders of the N.W.T.

Cryptocotyle lingua

This intestinal trematode, which appears to have been introduced to the Maritime Provinces of Canada, has spread both north and south. It is now found in the St. Lawrence estuary and the Labrador.

Records from the area under review include:

Anticosti Island, Gulf of St. Lawrence.
Cartwright, Labrador.
Flower Cove, Nfld.
Hebron, Labrador.
Hopedale, Labrador.

Alaria sp.

A species of Strigeid, which on the morphology of the egg and its resemblance to that found by us in actual trematodes, we refer to this genus, is recorded from:

Cumberland House, Sask.
Moosonee, James Bay, Ont.
Rupert House, James Bay, Que.
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens of a species of *Alaria* were recovered from a dog from Moosonee.

Miscellaneous Trematodes

Trematode eggs, which the writers were unable to identify because of their non-correspondence to published descriptions or to specimens collected from these regions, occurred in the faeces of dogs from:

Berens, Man.
Cambridge Bay, N.W.T.
Eskimo Point, N.W.T.
Fort Smith, N.W.T.
Port Harrison, Que.

Acanthocephala

A single species of Acanthocephala was recovered from a husky dog at Dundas, N.W.T. It has been identified by Prof. H. J. Van Cleave as ***Corynosoma semerme*** (personal communication), a species hitherto unrecorded from North America.

A NEW TREMATODE, *FIBRICOLA LARUEI*, FROM THE RACOON IN CANADA[1]

By M. J. Miller[2]

Abstract

A new trematode, *Fibricola laruei*, is described from the racoon in Canada.

The racoon (*Procyon lotor*) in the Province of Quebec was found to harbour an undescribed strigeid of the sub-family Alariinae. Its morphology showed it to be most closely related to members of the genus *Fibricola*, to which it is assigned, with the specific designation *laruei* sp. nov.

Description

Small, comparatively thin forms measuring from 0.7 to 1.17 mm. in length when mature; body divided into two parts, the lengths of the anterior and posterior parts being in the approximate ratio of 3 : 2; anterior part of body flattened with the edges inrolled ventrally and meeting medially near the junction of the two segments of the body (0.46 to 0.5 mm. wide); posterior segment narrow and cylindriform (0.22 to 0.3 mm. wide); terminal oral sucker slightly longer than broad (average size, 0.076 by 0.066 mm.); pharynx conspicuous, elongate, about the same length as the oral sucker and about two-thirds as broad; oesophagus slightly shorter than pharynx; intestinal caeca extend well into the posterior segment; ventral sucker usually broader than long, situated at the junction of the anterior and middle thirds of the body (average size, 0.026 by 0.033 mm.); muscular holdfast organ situated medially at the posterior end of the anterior segment (average size, 0.11 by 0.07 mm.); vitellaria composed of numerous small follicles confined to the anterior segment; testes two in number situated asymmetrically in the posterior segment, the posterior testis considerably broader, reniform in shape, the anterior testis situated immediately above the posterior one usually on the right, but occasionally on the left side of the body, roughly spherical in shape (average size posterior testis, 0.06 by 0.17 mm.; average diameter anterior testis, 0.13 mm.) ovary small, wider than long, above and partially overlapping anterior testis in a dorsal position (average size, 0.08 by 0.05 mm.); eggs large, rarely over two present in the uterus at one time (average size, 0.12 by 0.07 mm.).

Discussion

According to Dubois (2) the subfamily Alariinae contains five genera: *Alaria*, *Cynodiplostomum*, *Fibricola*, *Pharyngostomum*, and *Podospathalium*. The present form obviously does not belong to either *Pharyngostomum* or

[1] *Manuscript received March 4, 1940.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada, awarded through the National Committee on Fish Culture.

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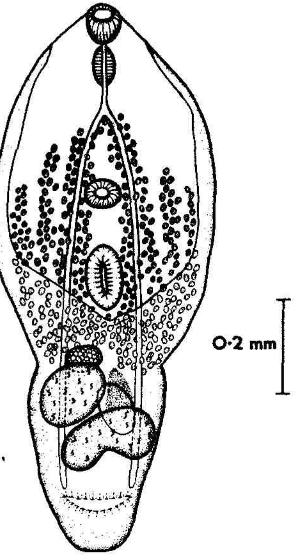

FIG. 1

Podospathalium. It differs from *Alaria* and *Cynodiplostomum* in that it has no pseudo-suckers or other appendages of any sort at the cephalic extremity. It differs from *Fibricola* in the character of the holdfast organ, which is elliptical in the present form and circular in *Fibricola*. With the exception of this character, however, its general morphology agrees very closely with that of the *Fibricola* species. The difference in shape of the holdfast organ does not appear to be sufficient reason for the creation of a new genus, and so the present form is assigned to the genus *Fibricola* and the generic definition amended to read as follows:

Alariinae with the body more or less distinctly divided into two parts; the cephalic extremity without sucker-like or other appendages; the holdfast organ either circular and one-third of the length of the anterior segment or elliptical and about one-fifth the length of the anterior segment; the posterior segment shorter than the anterior one; the ovary situated at the junction of the two segments; the posterior testis larger than the anterior one, which is asymmetrically developed opposite the Mhelis gland; no genital cone; shallow ***burs copulatrix*** *with a subterminal pore.*

Host: Racoon (*Procyon lotor*)

Location: Small intestine.

Locality: Argenteuil Co., Quebec, Canada. (It has also been found in racoons from other parts of Quebec.)

Types and paratypes in the helminthological collection, Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.

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A REVISION OF THE AMERICAN SPECIES OF *GONIA* MEIGEN (DIPTERA: TACHINIDAE)[1]

By Frank O. Morrison[2]

Abstract

Twenty American species of *Gonia* are separated on the basis of the shape of the anal forceps coupled with other characters. Four species, *G. grandipulvilli*, *G. discalis*, *G. albagenae*, and *G. tenuiforceps*, are described as new. The anal forceps are figured for each species. *G. brevipulvilli* Tothill is a synonym for *G. longiforceps* Tothill. A phylogenetic order and grouping of the species is based on the shape of the forceps, season of capture, and habitat. Complete taxonomic history of all categories is given.

Introduction

The tachinid genus, *Gonia* Meigen, has long been known to contain several distinct American species. Descriptions of at least 19 of these have been published. Determinations have been based on many different characters, of which those of the male genitalia have been favoured recently. It is, however, admittedly difficult, if not almost impossible, to describe in words slight differences in the shape or contour of such structures. Yet in no case has one of these characters been illustrated. As a result the accurate specific determination of *Gonia* has been practically impossible, and in almost all dipterous collections various species of this genus may be found under the name of *Gonia capitata* DeGeer, a European species of which the occurrence in America is very doubtful.

The recent extensive work on biological control has brought into prominence our various entomophagous parasites, and especially those parasitic on pests of economic importance. *Gonia* is known to be parasitic on lepidopterous larvae and has been held (17) to be an important control factor in damaging cutworm outbreaks in the grain-growing areas of Western Canada.

Townsend (21), discussing the habits of this and related insects that lay their microtype eggs on plant foliage, where they are ingested with the foliage by lepidopterous and other larvae, goes so far as to suggest that such parasites might be bred and distributed as a control measure for certain pests.

A clarification of the taxonomy of this group has been attempted in the present work by a study of the genital and other significant distinguishing characters. Where possible the work has been based on an examination of type material. A complete taxonomic bibliography of the genus and each species dealt with is included. Some synonomy is suggested, but it seems possible that, when further type material is available and especially when

[1] *Manuscript received January 19, 1940, and as revised May 9, 1940.*

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[2] *Lecturer, Department of Entomology, Macdonald College.*

European forms can be studied, further synonomy may be shown. Four new species are described and figured.

In approaching this problem an anatomical study of the male genitalia of *Gonia* was found imperative. Such a study forms the second part of this paper, to be published later. It was hoped that less easily exposed structures than those previously used might prove valuable in taxonomic work. Although no characters of taxonomic value were found, it became evident from the literature on the morphology of the male genitalia of Calyptrate Diptera that, though extensively used for taxonomy, the morphology of these structures is as yet poorly understood. The literature was carefully reviewed and synopsized. Considerations of space, however, make it inadvisable to include the synopsis. A table of synonomous morphological terms compiled from the literature is included, as are also the citations of the papers used and a detailed account of the anatomical study of the genital structures in *Gonia*, with some suggestions as to the possible homologies of the parts with those in other Diptera and with the parts of the male genitalia of more generalized insects. The taxonomy of Diptera in general cannot but be furthered by a more complete understanding of, and more uniform terminology for, the parts of these much used structures.

Historical Review

The genus *Gonia* was established by Meigen (9) in 1803, who later described 13 species (10). Curtis (5) designated *Musca capitata* DeGeer as the type of the genus. Desvoidy (6) described two genera, ***Rhedia*** and ***Reaumuria***, which were synonymized with *Gonia* by Coquillet (3). Desvoidy had wrongly supposed the term *Gonia* preoccupied in conchology. Two other generic descriptions published by Desvoidy (7), *Isomerya* and *Pissemya*, have been synonymized with *Gonia* Meigen by Schiner (14) and Brauer and Bergenstam (2) respectively. Townsend (21), in his as yet only partly published "Manual of Myiology", recognizes as separate genera, *Reaumuria* Desvoidy (type *Gonia ornata* Meigen) and *Rhedia* Desvoidy (type *Gonia atra* Meigen), as well as three American genera: *Cnephalogonia* Townsend (type *Gonia distincta* Smith), *Cystogonia* Townsend (type *Gonia turgida* Coquillet) and *Knabia* Townsend (type *Knabia hirsuta* Townsend), and at least one other exotic genus, *Phosphocephalops* Townsend (type *Gonia pallens* Williston), all of which would fall within *Gonia* Meigen as considered here. *Isomerya* Desvoidy he synonymized with *Gonia* Meigen s.s. and *Pissemya* Desvoidy with *Rhedia* Desvoidy. It is impossible, however, to determine from the volumes published to date the limits of Townsend's restricted genera or their synonomy with species considered in this paper. Further definition of these forms must await the publication of the additional volumes of his work containing the generic and specific descriptions.

Meigen and other European taxonomists depended largely on colour characters and the relative lengths of the aristal segments for specific determinations in this genus. The resulting descriptions are totally inadequate to allow any

comparison of our now known American species with the European forms on the basis of these descriptions alone.

Say (13) described the first known American species, *Gonia frontosa* Say, stressing colour. What is probably the same species was redescribed as *Gonia philadelphica* Macquart (8) and again as *Gonia albifrons* Walker (22). Williston (24) recognized the synonym of the above mentioned names, redescribed the species as *Gonia frontosa* Say, and described four new species, giving a key for the separation of the five species mentioned. He discarded the relative lengths of the aristal segments as a character variable in individuals and noted the colour of the antennae, colour of the thoracic vestiture, and length of the claws and pulvilli in the males.

Townsend (19) added another species, *Gonia sagax* Townsend.

Coquillet (3) reduced the previously described species to two, synonymizing *Gonia sagax* Townsend and *Gonia senilis* Williston, and synonymizing *Gonia frontosa* Say, *Gonia exul* Williston, and the European species *Gonia capitata* DeGeer, and described a third, new species, *Gonia turgida* Coquillet. *Gonia porca* Williston he recorded as "unrecognized".

Smith (15) described a new species, *Gonia distincta* Smith, basing his description on females only. In the following year Townsend (20) described a new, related genus, *Cnephalogonia* Townsend, with *Gonia distincta* Smith as the type species.

Strickland (17), working with cutworm outbreaks, found very different types of larvae of *Gonia* present. Tothill (18) revised the genus, largely on material reared by Strickland. Tothill's was the first classification based on male genitalia, the forceps of the genitalia being described but not figured. He recognized 17 species, 10 of which he described as new. *Gonia frontosa* Say, *Gonia sagax* Townsend, *Gonia senilis* Williston, *Gonia porca* Williston, and *Gonia sequax* Williston, he recognized as distinct species along with *Gonia distincta* Smith and *Gonia turgida* Coquillet.

Reinhard (12) described an additional new species, *Gonia texensis* Reinhard, again describing but not illustrating the male genitalia.

Present Revision of the Genus

The writer commenced work on this genus at the University of Alberta, where a considerable collection of *Gonia* had accumulated. This was augmented by specimens from the University of Montana, the Dominion Entomological Laboratory at Saskatoon, Saskatchewan, and material collected by the writer and others at Macdonald College, Province of Quebec. Dr. McDunnough, Department of Agriculture, Ottawa, kindly allowed the examination of Tothill's type material and of several hundreds of specimens in the Canadian National Collection.

Early in the investigation it became evident that, at least at present, accurate determination of female material must remain impossible. It is the hope of the writer to investigate, at a later date, the connection between the species

as here determined on male characters, and the different types of larvae (17). This, together with a study of the female genitalia, might lead to the discovery of means of classifying females.

For the purpose of the present paper, males only are considered except where the female definitely associated with these males is known. The key is based on combinations of characters of the genitalia and of other structures. The only portion of the male genitalia showing characters of taxonomic value is the anal forceps (Fig. 1). This compound structure is normally folded underneath the abdomen and concealed in the genital pouch beneath the lobes of the fifth sternite. From this position it must be extruded with a hooked needle in order that lateral and dorsal views may be observed. The terms "dorsal" and "ventral" as used in this paper refer to the forceps in this extended position. The outline of the forceps in the dorsal and lateral views offers the best characters for separating the species.

The drawings presented here were prepared from genitalia (of type materia where possible) first removed from the specimen, then treated for from 24 to 36 hr. in cold 10% potassium hydroxide, and kept in vials of 40% alcohol and 10% glycerine solution. They were examined in glycerine with the aid of a binocular microscope. A camera lucida was attached to one tube of the microscope to aid in securing the outline. Permanent mounts were made in balsam or De Faure's solution, but this is not advisable as it prevents any further moving of the material. For purposes of determination in later work, it did not prove necessary to dissect out the genitalia from the body. They were merely extruded to reveal the forceps and dried in that position. A word of warning should be given here pointing out that unless the observations are made from perfectly similar positions in all cases (i.e., lateral and dorsal views), appearances may be very misleading. The dorsal view is difficult to get in such a way that the two sides of the forceps are symmetrical. Thus an apparent asymmetry is noticeable in some of the diagrams presented in this paper.

In many species the appearance of the genitalia is not sufficiently distinctive to serve alone as a distinguishing character. Other morphological structures must then be used.

The key given has been built with one chief thought in mind; i.e., that it should be usable and make exact separation of the species possible. For this reason the species key out separately and not into the groups of related species that are discussed later.

Gonia Meigen

1803 GONIA Meigen (9). 1826 GONIA Meigen (10), (13 spp. described). 1830 RHEDIA Desvoidy (6) (Synonymy by Coquillet). 1830 REAUMURIA Desvoidy (7), (Synonymy by Coquillet). 1834 GONIA Meigen (5), (*Musca capitata* DeGeer, designated as type). 1849 GONIA Meigen (22). 1851 ISOMERYA Desvoidy (7), (Synonymy by Schiner). 1851 PISSEMYA Desvoidy (7), (Synonymy by Brauer and Bergenstam). 1862 GONIA

Meigen (14). 1878 GONIA Meigen (11). 1887 GONIA Meigen (24), (Desc. and table of spp.). 1894 GONIA Meigen (16), (Discussed generic limits). 1897 GONIA Meigen (3). 1924 GONIA Meigen (18). 1934 GONIA Meigen (21), (Greatly restricted).

Gonia Meigen is easily separated from other genera of Tachinids by the following combination of characters: head very much inflated (except in *G. distincta* Smith), yellowish except on the occiput and the eyes, pollinose with the front above largely translucent and more than twice as wide as either eye in both sexes (wider in females), the frontal vitta not strikingly darker in ground colour, ocellar bristles strong and curved backward, orbitals present in both sexes, eyes bare, antennae black or yellow, the penultimate aristal segment always more than three times as long as wide, usually a strong bend at the junction of the second and third aristal segments giving the arista a geniculate appearance, facial ridges with only a few bristles at their bases, parafacials with hair or bristles; propleura bare; lower lobes of squamae bare above; infrasquamal setulae absent. It is to be noted that the generic keys (3, 25) would eliminate *Gonia setigera* Tothill, in which the first vein is setose, from this genus. The earlier generic descriptions also list the palpi as yellow but in the *fissiforceps* group specimens show palpi with varying degrees of infuscation to dark brown. Tothill describes the first two antennal segments as yellow in all cases. This is not quite correct as frequently only a slight reddish-yellow colour on the apical portions of these segments is obvious, the segments being otherwise dark.

Key to the Males of *Gonia* Meigen Found in America North of Mexico

(For the purposes of this key the genitalia are considered as extruded and extended until the forceps point backward in the direction of the long axis of the body. The terms dorsal, ventral, lateral, "turned up", and "turned down" refer to the structures in this position. Figs. 1 to 22 are lateral views of the forceps; Figs. 1a to 22a, dorsal views.)

1. First, second, and third segments of antennae entirely yellow, aristae black or mostly yellow....................................2.
   At least most of the third antennal segments as well as the aristae dark in ground colour....................................3.
2. Aristae mostly yellow, genal hairs white; forceps thick, straight on the ventral edge when viewed in profile, not over twice as long as the median width; as in Figs. 13, 13a..........(No. 13) *senilis* Williston.
   Aristae black, genal hairs dark; forceps three times as long as their median breadth, ventral edge convex toward the apex, as in Figs. 2, 2a....................................(No. 2) *sagax* Townsend.
3. First wing vein bare with at the most one or two setae..............4.
   First wing vein setose on the dorsal side, basally, half way to the first cross vein; forceps as in Figs. 10, 10a..........(No. 10) *setigera* Tothill.

4. Forceps without a conspicuous dorsal carina . . . . 5.
   Forceps with a strongly developed carina as deep as the forceps are thick and extending for ¾ the length of forceps . . . (No. 21) *carinata* Tothill.
5. Pile on the occiput white . . . . 6.
   Pile on the occiput brown; forceps as in Figs. 3, 3a . . . . (No. 3) *fuscicollis* Tothill.
6. Pleurae without yellow pile; lobes of fifth sternite black; genal pile light or dark . . . . 7.
   Pleurae with yellow pile; lobes of fifth sternite yellow; genal pile fine and yellow; forceps as in Figs. 16, 16a . . . . (No. 16) *porca* Williston.
7. Lobes of the fifth sternite excised laterally (as in Fig. 18b); abdomen shining black, with dorsal segmental bases narrowly or not at all pollinose . . . . 8.
   Lobes of the fifth sternite not excised laterally; abdomen frequently reddish on the sides, dorsal segmental bases often broadly pollinose . . . 9.
8. Forceps more than half as deep as wide at the base of the apical cleft, ventral edge almost straight, dorsal convexity extending only about one-third of the distance to the apex, which is blunt; in dorsal view with the apical cleft extending beyond the middle, as in Figs. 18, 18a . . . . (No. 18) *fissiforceps* Tothill.
   Forceps at the base of the apical cleft more than half as deep as wide, ventral edge almost straight but the dorsal convexity extending almost one-half the length of the forceps, apex blunt, apical cleft not extending beyond the middle, as in Figs. 20, 20a . . . . (No. 20) *yukonensis* Tothill.
   Forceps at the base of the apical cleft not half as deep as wide, ventral edge curved ventrally at the apex, which is almost hook-like, as in Figs. 19, 19a . . . . (No. 19) *tenuiforceps* new species.
9. Forceps almost as deep as long, dorsally with a subapical tuft of long black setae, ventrally with a cushion of yellowish setae in the hollow between the apical lobes, as in Figs. 17, 17a . . (No. 17) *texensis* Reinhard.
   Forceps much longer than deep, no cushion of yellowish setae between the apical lobes ventrally . . . . 10.
10. Genal hairs light in colour . . . . 11.
    Genal hairs dark in colour . . . . 12.
11. Forceps broad as in Fig. 12a, ventral edge slightly curved dorsally at the apex as in Fig. 12, abdomen mostly yellow with a narrow dorsal dark stripe; abdominal venter yellow except at the base and apex; eastern species . . . . (No. 12) *sequax* Williston.
    Forceps narrow as in Fig. 9a, ventral edge straight as in Fig. 9, abdomen mostly yellow with dark dorsal stripe prominent; venter with a longitudinal black stripe . . . . (No. 9) *albagenae* new species

12. Parafacials narrower than the greatest width of the eye; pulvilli and ungues long as in Fig. 14b; forceps with dorsal convexity reaching almost to the apex, as in Figs. 14, 14a......(No. 14) *distincta* Smith.

13. Parafacials unusually wide with numerous long black setae next to the eyes and directed medially, setal vestiture of front, parafacials, and genae generally denser, longer, and darker than in any other species; forceps with a straight ventral edge, dorsal convexity not conspicuous, as in Figs. 11, 11a........................(No. 11) *turgida* Coquillet.

Parafacials as usual, with scattered black setae mostly short; forceps with ventral edge curved or if straight forceps are short or have a conspicuous dorsal convexity..................................14.

14. Forceps short with a straight ventral edge, with or without a strong dorsal convexity, rarely with the apex slightly dilated dorsally in *breviforceps*..15.

Forceps short with a strongly curved ventral edge or longer with a very slightly to strongly curved ventral edge, the apex in profile always dilated dorsally, knob-like....................................16.

15. Forceps short, ventral edge straight, dorsal convexity conspicuous and extending about four-fifths of the distance to the apex, as in Figs. 15, 15a; pulvilli as long as the last tarsal segment; ungues slender, as long as pulvilli, yellowish basally, as in Fig. 15b....................
(No. 15) *longipulvilli* Tothill

Forceps short, and small, ventral edge straight, apex rarely slightly dilated dorsally, as in Figs. 8, 8a; pulvilli and ungues distinctly shorter than the last tarsomere..................(No. 8) *breviforceps* Tothill.

16. Forceps 2 to 4 times as long as deep, ventral edge strongly curved, apex distinctly knob-like as in Figs. 1, 1a, small dark species; 7 mm. to 10 mm. long; sides of abdomen sometimes reddish................
(No. 1) *frontosa* Say.

Forceps 5 to 8 times as long as deep, ventral edge almost straight to strongly curved, larger species; 9 mm. to 12 mm. long............17.

17. Forceps in dorsal view triangular, tapering from the base to the apex, as in Figs. 4, 4a; pulvilli and ungues shorter than the last tarsomere, as in Fig. 4b................................(No. 4) *aldrichi* Tothill.

Forceps in dorsal view continuing wide from the base or expanding, then tapering rapidly near the apex, as in Figs. 5, 5a; pulvilli and ungues longer than the last tarsomere, as in Fig. 5b......................
(No. 5) *grandipulvilli* new species.

Forceps in dorsal view tapering rapidly near the base, then extending long and narrow with almost parallel sides to the apex, as in Fig. 7a..18.

18. Dorsal pile on abdomen long and erect; forceps as in Figs. 7, 7a........
(No. 7) *discalis* new species.

Dorsal pile on abdomen short and recumbent; forceps as in *discalis* (Figs. 6, 6a)............................(No. 6) *longiforceps* Tothill. (= *brevipulvilli* Tothill).

1. *G. frontosa* Say

1829 *Gonia frontosa* Say (13). 1840 *Gonia philadelphica* Macquart (8), (Described from Philadelphia). 1849 *Gonia albifrons* Walker (22), (Described from the Hudson's Bay Territory). 1878 *Gonia frontosa* Say (11). 1887 *Gonia frontosa* Say (24), (Redescribed = *G. philadelphica* Macq., = *G. albifrons* Walk.). 1897 *Gonia capitata* (DeGeer) Coquillet (3), (Probably incorrect in synonymyzing *frontosa* with the European *capitata*) = *G. philadelphica* Macquart = *G. albifrons* Walker = *G. exul* Williston (Probably wrongly) = *G. sequax* Williston (Probably wrongly). 1905 *Gonia capitata* (DeGeer) (7) (Follows Coquillet in synonymy). 1924 *Gonia frontosa* Say, Tothill (18) (Redescribed—neotype named—no synonymy mentioned—distinguished from *G. sequax* Willst. *G. exul* Willst. not mentioned).

There seems to be little doubt of the synonymy of Say's species with *Gonia albifrons* Walker and *Gonia philadelphica* Macquart. The synonymy with *G. sequax* Williston and *G. exul* Williston suggested by Coquillet and followed by Aldrich seems improbable in view of the original description of *sequax* "with abdomen reddish yellow" and of *exul*, "with claws and pulvilli of male large." The synonymy of this species with the European *G. capitata* (DeGeer) is also doubtful. A specimen in the Canadian National Collection from Europe, and bearing the label *G. capitata* (DeGeer), determined by Bezzi, has very different forceps (Figs. 22, 22a). Williston (24), when he redescribed the species from Minnesota males, appears to have had this species, but the New England, North Carolina, and California material which he mentions was evidently a mixture of species with more red coloration on the abdomen, yellowish genal hairs, etc.

Considering the widespread distribution of this species it is probably the oldest form, phylogenetically speaking.

Type locality: upper Missouri River.

Distribution: widespread throughout Canada and United States.

Neotype: No. 785. Canadian National Collection.

2. *G. sagax* Townsend

1893 *Gonia sagax* Townsend (19). 1897 *Gonia senilis* Williston, Coquillet (3), (Synonymy probably incorrect). 1905 *Gonia senilis* Williston (1), (follows Coquillet). 1924 *Gonia sagax* Townsend (18), (Redescribed), Neotype named from Middlesex County, N.J. (Figs. 2, 2a).

The Tothill neotype of this species was captured in April, in Middlesex County, N.J. Length 10 mm., width 4 mm. Abdominal segments three and four are slightly reddish on the sides; the antennae are all yellow except the aristae which are brownish black (a feature that checks with Townsend's original description); genal hairs dark; pulvilli short, small, oval; ungues

not more than two-thirds the length of the last tarsomere; face and frontal vitta strongly yellow pollinose. Tothill has described the forceps as, "of medium length and not blunt as in *G. senilis* Williston, about three times as long as their median width and much narrower across the base of the apical cleft than at the middle."

In the Canadian National Collection is a female specimen from Aylmer, Que., 14, v, 1925, taken by G. S. Walley, with the same colour markings but very small, being barely 8 mm. long and appearing at first glance to be *frontosa* Say.

Type locality: Ames, Iowa.

Distribution: Iowa, New Jersey, Quebec (?).

Neotype: in the Canadian National Collection.

3. *G. fuscicollis* Tot.

1924 *Gonia fuscicollis* Tothill (18). (Figs. 3, 3a)

The only specimen of this species available was the male paratype, La Fayette, Ind. April, 1916 (J. M. Aldrich) in the Canadian National Collection. This specimen, Tothill tells us, bears the same data as the holotype in the United States National Museum. The general appearance of this small dark specimen (9 mm. by 4 mm.) is strikingly like *frontosa* Say as is the ventrally arcuate line of the forceps. The ventral line of the forceps is more convex than in any *frontosa* observed. The occipital pile is sparse, long, and brown.

A female from Rosthern, Saskatchewan, May 4, 1925, (K. M. King), in the Canadian National Collection, has a light brownish yellow occipital pile, but the abdomen is broadly red on the sides, the colour spreading ventrally to leave only a narrow median longitudinal dark stripe.

The occipital pile of the other species though generally white often shows varying degrees of yellowing to dark yellow, especially in specimens reared in captivity and specimens which appear to have been "wet" at some time. Further collecting may prove this species good or doubtful; at present it must be maintained on the strength of the type and paratype.

Type locality: La Fayette, Ind.

Distribution: ?

Type: United States National Museum.

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frontosa group

The three above named species have short forceps with arcuate ventral edges and appear to form a closely related group. All specimens examined have been captured in April, May, or early June.

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4. *G. aldrichi* Tothill

1924 *Gonia aldrichi* Tothill (18). (Figs. 4, 4a)

Among the many paratypes of Tothill's species *aldrichi* are two distinct species. His own description of the forceps, "unusually robust, being both long and wide. About three times as long as the median width and almost triangular in dorsal view the equal sides tapering from the base to tip. The dorsoventral flattening is very marked," is descriptive of the type, and sufficient to separate out this species. Add to this that the ungues and pulvilli in the male are distinctly shorter than the last tarsomere, and no difficulty in separation from the following species should arise.

Tothill records *aldrichi* as bred by Strickland from *Euxoa ochrogaster* and *Agrotis orthogonia* (Lepidoptera: Noctuidae). Adults are collected in April and May.

Type locality: Coaldale, Alberta.

Distribution: across Canada and the northern United States.

Type: No. 786, Canadian National Collection.

5. *G. grandipulvilli* new species (Figs. 5, 5a, 5b)

Male: length, 9 to 12 mm.; width, 4 to 5 mm. A comparatively large, dark species. Face and front light yellow with a silvery sheen in some lights, front above with a waxy appearance on the sides; lines of the face and front in profile almost straight and meeting at an angle just less than a right angle; parafacials narrower at the oral margin than at the vibrissal base, at the narrowest point wider than the greatest eye width, with an uneven double or triple row of black setae (which vary greatly in size) along the inner edge of the eye and separated from the usual row of graduated bristles along the frontal suture by a bare area. Genae less than one-half the eye height with numerous slender dark setae; antennae blackish with a greyish sheen in some lights; second antennal segment slightly reddish apically, with short dorsal setae, third segment five to seven times as long as the second; pile on the occiput white; palpi yellow. Dorsum of the thorax with five broad, longitudinal pollinose lines; scutellum yellowish at least apically, frequently dark basally, some yellow on the humeri, legs black. Pulvilli and ungues distinctly longer than the last tarsomere; the pulvilli broad and white to yellowish brown; ungues black, heavy, and extended almost at right angles to the tarsomere; tarsomeres somewhat broad and flat; wings clear with but very slight costal infuscation, first vein (R_1) bare above, third vein (R_{4+5}) with a group of setae above and below at the point where (R_{2+3}) and (R_{4+5}) separate.

Abdomen with reddish patches on the sides of segments 2 and 3 (this reddish colour though involving considerable of the abdomen in a few specimens examined is characteristically limited to separate patches of varying size on the segments mentioned). Dorsally the abdominal tergites narrowly

to broadly pollinose basally, the pollen spreading out laterally on the fifth segment to cover the sides. Dorsal vestiture short and semi-erect.

Forceps in lateral view resemble those of *aldrichi*, strong, long, and flattened; ventral edge slightly curved, apical dorsal convexity evident, dorsally with sides parallel or divaricating more than half way to the apex, then tapering rapidly to the blunt point, outline much less nearly triangular than in *aldrichi*.

Type:—Edmonton, Alta. 25/4/23. F. S. Carr, No. 5032 Canadian National Collection, (Genitalia, vial No. 27).

Paratypes:—Edmonton, Alta.: as type (vial No. 25); 2, v, 1937, F. O. Morrison; 4, v, 1937, E. H. Strickland. Lethbridge, Alta.: ? Seamans and Strickland; April 17, 1925,—April 11, 1926, 2 specimens, H. E. Gray; May 3, 1923,—April 18, 1923, H. L. Seamans. Saskatoon, Sask.: April 20, 1937, 3 specimens, A. P. Arnason; 15 iv, 1915, ?; 8, v, 1923,—April 26, 1924, 3 specimens,—22, iv, 1923,—May 11, 1925,—10, v, 1923—April 29, 1924, K. M. King; May 11, 1923,—3, v, 1923, N. J. Atkinson. Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Bozeman, Montana: June 2, (192?), ? (vial No. 53). Toronto, Ont.: iv, 25, 1906. Merivale, Ont.: 3, v, 1938, 2 specimens, G. E. Shewell. Maple Sap, 1, May, 1921. F'ton: 27, iv, 13. Vernon, B.C.: 28, iv, 1924, 2 specimens, E. R. Buckell. Kaslo, B.C.: 10, iv, 1907, Cockle (vial No. 35). Montreal, Que.: 17, v, 1915, J. I. Beaulne. The following are among Tothill's paratypes of *aldrichi* in the Canadian National Collection.

Ottawa: 15, April, 1915 (Genitalia slide No. 12); 1905, James Fletcher; 22, iv, 1906, 2 specimens,—1, 4, 1906, W. Metcalfe; 18, iv, 1915, A. E. Kellet; and one labelled 0.15.4. 1889. O. Ottawa. Jordan, Ont.: 20, iv, 1919, C. H. Curran. One not labelled. Mussellshell, Mont.: 5, 12, 1917, Mont. Exp. Sta. Mac. Coll.: 11/4/1915. Toronto, Can.: 16, iv, 1895, E. M. Walker; April 21, 1897? Hull, Que.: 17, April; 15 ?, 2 specimens. Logan Ut. Jn. 1915. H. R. Hogan.

6. *G. longiforceps* Tothill*

1924 *Gonia longiforceps* Tothill (18) (= *Gonia brevipulvilli* Tothill). (Figs. 6, 6a)

The figures show Tothill's description of the forceps in this species very accurately. "Forceps long and slender being about five times as long as the median width. In dorsal view the sides taper abruptly at the base and then continue almost parallel to the apex. Viewed in profile the dorsal declevity starts well beyond the middle and the ventral edge is arcuate."

* NOTE: *Names given by Tothill and ending in the word "—forceps" were published in this form. The type labels, however, carry the Latin genitive form "—forcipis". The names stand as published and the same form has been adopted for new species names involving this word.*

Where the word "forceps" itself occurs, apart from names (in the text and key) it has been used in the anglicised form and considered as plural, comparable to the word "scissors". The singular and plural Latin forms "forceps" and "forcipes" seem unnecessarily awkward.

There appears to be no difference between the type and paratypes of this species and those of *Gonia brevipulvilli* Tothill, which is here made a synonym.

Among the paratypes of this species and other material previously grouped here occur specimens that have been segregated to form the new species, *G. discalis*. This species differs from *G. longiforceps* in having the dorsal vestiture of the abdomen long, the setae often attaining a length equal to one-half the length of the segments, and borne almost erect; whereas the dorsal abdominal vestiture of *longiforceps* is short and semi-recumbent.

Gonia longiforceps is recorded as bred from *Agrotis orthogonia* (Noctuidae: Lepidoptera).

Type locality: Lethbridge, Alberta.

Distribution: across Canada and the United States.

Type: No. 784 Canadian National Collection.

7. *G. discalis* new species (Figs. 7, 7a)

Male, length 9 to 13 mm. Dark species. Face and front light yellow, with a silvery sheen in some lights; the front from above has a waxy appearance on the sides; parafacials only slightly narrower at the oral margin than at the vibrissal bases, narrowest width greater than the greatest eye width, with a double or triple row of black setae along the inner edge of the eye, others scattered or in broken rows inside these and the usual short row of heavier bristles along the lower edge of the frontal suture, these bristles graduated in length from the shortest at the top of the row to the longest outside and considerably above the oral vibrissae; genae about half the eye height with a vestiture of slender black setae; antennae blackish often with a greyish sheen in some lights; second antennal segment yellowish with short, heavy, dorsal setae; third segment five to seven times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum and small area preceding it yellow; some yellow on humeri. Legs black. Pulvilli and ungues distinctly shorter than last tarsomere. Wings slightly infuscated basally on the dorsal edge.

Abdomen shining black, segments 3, 4, and 5 pollinose basally, red coloration occurs on segments 3 and 4 and sometimes 5 in varying amounts. The dorsal abdominal vestiture on segments 3 and 4 is long and erect or almost erect. The setae reach a length equal to half the length of these segments.

Forceps similar to those of *longiforceps*, dorsally, wide at the base, tapering rapidly then extending with almost parallel sides to the apex. In lateral view the basal dorsal convexity extends less than one-half the length of the forceps; the apex is expanded dorsally, knob-like.

Type:—Nicola, B.C.: 28, iv, 1922, P. N. Vroom, No. 5003 Canadian National Collection.

Paratypes:—Nicola, B.C.: 23, v, 1922, (vial No. 114),—29, v, 1922, P. N. Vroom. Vernon, B.C.: 13, 4, 1927,—20, iv, 1927, I. J. Ward. Agassiz, B.C.: 29, iii, 1924,—6, iv, 1922. R. Glendenning. Cranbrook, B.C.: 11, v, 1922, 3 specimens,—19, v, 1922,—10, v, 1922,—12, v, 1922,—8, v, 1922, C. B. D. Garrett. Penticton, B.C.: 12, iv, 1927, E. R. Buckell. Copper Mtn., B.C.: 8, iv, 1928. G. Stace Smith. Toronto, Ont.: 13, 4, 1905. Ottawa, Ont.: 22, iv, 1906, W. Metcalfe (Slide No. 13). Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Saskatoon, Sask.: 27, iv, 1923, K. M. King. Lethbridge, Alta.: April 30, 1915, E. H. Strickland.

The following paratypes of *longiforceps* Tothill are in the Canadian National Collection.

Vancouver, B.C.: 10, viii, 1907,—1, 4, 1916, R. S. Sherman. Treesbank, Man.: 17, iv, 1908, J. B. Wallis. Aweme, Man.: 16, v, 1921, N. Criddle. Chilicotin, B.C.: 24, iv, 1920, E. R. Buckell (Genitalia vial No. 115).

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aldrichi group

Gonia aldrichi, *G. grandipulvilli*, *G. longiforceps*, and *G. discalis* form a second group of species all closely related. The dark coloration and arcuate ventral edge and apical swelling of the forceps suggest a close relationship to the first or *frontosa* group. In general the forceps in this group are longer and stronger than in the first and the flies are larger. Specimens have been captured throughout the breadth of the United States and Canada during the spring months.

The third or *breviforceps* group, which follows, is also taken in the spring, but the ventral edge of the forceps is straight and the distribution of the species is largely limited to central and western Canada and the United States.

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8. *G. breviforceps* Tothill

1924 *Gonia breviforceps* Tothill, (18). (Figs. 8, 8a)

From this species as described by Tothill the following new species, *G. albagenae*, has been separated. The new species includes one paratype of *breviforceps* Tothill, Vernon, B.C., 25, v, 1919. W. B. Anderson, genitalia slide No. 14, Canadian National Collection. *G. breviforceps* has distinctly dark setae on the genae while *G. albagenae* has a vestiture of fine white setae on the genae except for a few darker hairs at the lower edge in some specimens. In general the parafacials are slightly narrower in *breviforceps* Tothill.

G. breviforceps has been bred from *Euxoa ochrogaster* both in Alberta and Saskatchewan.

Type locality: Lethbridge, Alberta.

Distribution: British Columbia, Montana, California, Colorado.

Type: No. 788, Canadian National Collection.

9. *G. albogenae* new species (Figs. 9, 9a)

Male: length 9.5 to 11.5 mm. Specimens usually show considerable red, but dark ones occur. Face and front light yellow, with a silvery sheen in some lights; front from above has a waxy appearance on the sides; parafacials narrowing very little from the aristal bases to the vibrissae, at the narrowest point about equal in width to the greatest eye width, with an uneven double or triple row of slender black setae along the inner edge of the eye and others scattered or in broken rows inside these, the usual short row of graduated bristles along the frontal suture; genae about half eye height, covered with sparse fine white setae, at the most a few dark setae occur next to the basal row of bristles; antennae blackish with a greyish sheen in some lights; second segment yellowish with short heavy dorsal setae, third segment 5 to 7 times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum, small lateral areas preceding it, and parts of humeri yellowish. Legs black. Pulvilli and ungues distinctly shorter than last tarsomere. Wings clear with very slight costal infuscation.

Abdomen usually broadly reddish on the sides of segments 3 to 4, the coloured areas sometimes extending to other segments and ventrally, leaving only basal and apical dark rings connected by a narrow dark stripe dorsally or by dark dorsal and ventral stripes. Lobes of fifth sternite dark. Dorsal abdominal vestiture short and semi-erect.

The forceps resemble those of *breviforceps*, short and straight on the ventral edge, little dorsal convexity, dorsally with almost parallel sides for the greater part of their length.

A female (Vaisseaux, B.C., 14, vi, 1919, W. B. Anderson), apparently of the same species has similar body markings and white genal hairs.

Type:—Penticton, B.C.: 12, iv, 1927, E. R. Buckell, No. 5034 Canadian National Collection.

Paratypes:—Penticton, B.C.: 21, iv, 1927, E. R. Buckell (Genitalia vial No. 113). Lillooet, B.C.: ? A. W. A. McPhair. Naramata, B.C.: 2, v, 1919, E. R. Buckell (Genitalia vial No. 132). A paratype of *breviforceps* Tothill in the Canadian National Collection. Vernon, B.C.: 25, v, 1919. W. B. Anderson (Genitalia slide No. 14).

10. *G. setigera* Tothill

1924 *Gonia setigera* Tothill, (18). (Figs. 10, 10a)

Drawings were prepared from the one male paratype (No. 781 Canadian National Collection) with same data as the type. Two male specimens, one from Penticton, B.C., 21, iv, 1927, E. R. Buckell, with forceps exactly as in the paratype and a second from Oliver, B.C., 23, iv, 1927, (Genitalia vial No. 131), E. R. Buckell, of which the forceps show a more evident dorsal convexity but are otherwise similar, are in the Canadian National Collection. Males and females of this species are easily distinguishable by the numerous

setae on the first vein (R_1). The third vein (R_{4+5}) usually bears a greater number of setae than in any other species. As was pointed out in dealing with the generic limits of *Gonia* the generic keys of Williston and Coquillet exclude this species. In examining other species one specimen was noticed with one seta dorsally on the first vein of one wing only.

Type locality: Essex, Mass.

Distribution: Massachusetts, California, British Columbia.

Type: in the Museum of the Boston Society of Natural History.

11. *G. turgida* Coquillet

1897 *Gonia turgida* Coquillet, (3). 1924 *Gonia turgida* Coquillet, (18). 1936 *Cystogonia turgida* (Coq.) Townsend, (21). (Type of new genus *Cystogonia* Townsend). (Figs. 11, 11a).

Drawings were made from one of several male specimens from Idaho, May 2, 1919, E. H. Quales, in the Canadian National Collection, probably placed in this species by Tothill who redescribed the species from a male taken by E. G. Holt at Round Mountain, Nevada, 6,300 ft. (The writer was unable to locate this specimen.) Tothill had his specimen compared with the type by Aldrich, who mentions the striking colour pattern of the type. Among the material received from Saskatoon are two specimens, one from Three Mile Creek, Sask., 23, v, 1921, A. E. Cameron, and one labelled Mortlach, 31, v, 1909, which are evidently the same species. The original description of Coquillet, "the front near the eyes densely covered with rather long bristly hairs, sides of face each one and one-half times as wide as the median depression, densely covered with rather long black bristly hairs which are less numerous along the facial ridge," is sufficient to assure the identity of these specimens. The width of the parafacials compared to the eye width as used in Tothill's key is, alone, however, totally inadequate as a determining character.

Type locality: Los Angeles, California.

Distribution: California, Idaho, Saskatchewan.

Type: No. 3640 United States National Museum.

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breviforceps group

The writer considers the straight ventral edge and general shape of the forceps in this group to be indicative of close relationship. The body coloration is characterized by a distinct dorsal, longitudinal dark stripe on the abdomen. Frequently a ventral dark stripe is also present.

There appears to be a decided break between the three groups of species described above; i.e., the *frontosa, aldrichi,* and *breviforceps* groups and the two groups to follow. Adults of all species dealt with up to this point are collected in the spring months, while all those to follow are found in the late summer. This suggests a different host relationship and means of spending

the winter. No host records of the following species are known to the writer. Moreover, much wider variations in genital and other characters occur among the following forms. They are, as far as we have evidence, to date, generally more restricted in their habitats as well as specialized in genital structures.

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12. *G. sequax* Williston

1887 *Gonia sequax* Williston (24). 1897 *Gonia capitata* (DeGeer), Coquillet (3). = *Gonia frontosa* Say, = *Gonia philadelphica* Macquart, = *Gonia albifrons* Walker, = *Gonia exul* Williston (probably incorrect). 1905 *Gonia capitata* (DeGeer) (1) (follows Coquillet). 1924 *Gonia sequax* Williston (18) (redescribed). (Figs. 12, 12a).

The drawings were prepared from a male specimen from Jordan, Ont., 23, viii, 1915, C. H. Curran, in the Canadian National Collection. This locality is mentioned by Tothill, who no doubt examined this specimen. Four male and five female specimens were taken by the writer and his associates at Macdonald College during August and September, 1938. It is not clear whether Tothill examined the type material of *sequax*, but the large amount of yellow on the body stressed by Williston in his original description together with the short pulvilli make this determination fairly certain. Females taken at Macdonald College show a more widespread darkening on the dorsal surface of the abdomen than do the males which have a very narrow continuous or broken dorsal, dark stripe widening out at the base and apex of the abdomen. No ventral longitudinal dark stripe is present. The genal hairs are yellow.

Synonymy with *G. frontosa* Say can be discarded on colour, size, colour of genal hairs, and the appearance of the forceps. Synonymy with *G. capitata* (DeGeer) is similarly denied by the light colour and the yellow genal hairs. The forceps show a somewhat weaker dorsal convexity than do those of the specimen determined as *G. capitata* (DeGeer) by Bezzi (See Figs. 22, 22a) but are otherwise similar.

Tothill makes no mention of *G. exul* Williston. Coquillet and Aldrich synonymized this species with *sequax* Williston. It seems probable that the female described as *exul* was the female of *sequax*. It is not possible to be certain of this since females cannot be separated as definitely as males. However, this description fits females taken at Macdonald College along with males of *sequax*. The California males with long pulvilli mentioned by Williston and considered conspecific with the described females were most probably *G. longipulvilli* Tothill, while the North Park specimen with narrow parafacials was *G. distincta* Smith.

Type locality: California.

Distribution: California, Colorado, Texas, Connecticut, Massachusetts, British Columbia, Ontario.

Type: ?

13. *G. senilis* Williston

1887 *Gonia senilis* Williston (24). 1897 *Gonia senilis* Williston, Coquillet (3), = *Gonia sagax* Townsend (synonymy probably incorrect). 1905 *Gonia senilis* Williston (1) (follows Coquillet). 1924 *Gonia senilis* Williston, Tothill, (18), (redescribed as distinct from *sagax* Townsend). (Figs. 13, 13a).

Drawings were prepared from a specimen in the Canadian National Collection labelled, Oak Grove, Virginia, Fla., Daucus, 2, viii, ?, C. H. Townsend. This locality is mentioned by Tothill in his redescription of this species. Among other specimens examined were: one male from Severn, Ont., 16, 6, 1925, C. H. Curran; one male from College Park, Indiana, W. R. Walton; and two females apparently of the same species from Robinson, Delaware Co., Iowa, vii, 24, 1924, N. K. Bigelow. The suggested synonymy of this species with *sagax* Townsend is incorrect if the specimens available are accurately determined. The differences in the forceps, which are shorter and much broader in *senilis* Williston, are evident from the diagrams. Tothill separated the two species on the presence of dark setae on the genae in *sagax* and yellow setae in *senilis*. In the male from Severn, Ont., and one female from Delaware, at least numerous of the basal genal hairs are dark in some lights. The colour of the aristae, which in the original descriptions is given as orange yellow except at the tip in *senilis* and as brown in *sagax*, appears to be a good character but should be borne out by the forceps.

The differences in the forceps together with the fact that *sagax* has been captured in the spring months and *senilis* in the summer has led the writer to adopt the grouping in this paper and separate these two species so widely in spite of the similar colour of the third antennal segments.

Type locality: Western Kansas.

Distribution: Indiana, Virginia, New Jersey, Georgia, Florida, Ontario.

Type: ?

14. *G. distincta* Smith

1915 *Gonia distincta* Smith (15). 1916 *Gonia distincta* Smith, Townsend (20), (made the type of a new genus *Cnephalogonia* Townsend). 1924 *Gonia distincta* Smith, Tothill (18). (Figs. 14, 14a, 14b).

Gonia distincta Smith was described from three female specimens and the original description, except for the colour, gives us little information of specific value, especially as the specimens were female. Townsend, making this the type of his new genus, adds: "Female. Front not swollen . . . no median marginal macrochaetae on the first* abdominal segment; no closely set marginal machrochaetae on the third* segment. Parafacials below not over one-half greatest eye width, widening above to nearly eye width at base of antennae. Front marginal macrochaetae of parafacials sparse, few and weak."

* *"First" and "third" refer to the "second" and "fourth" segments respectively, as understood in the second part of this paper.*

Tothill redescribed the species from five males and two females, one of the females having been compared with the type described by Mr. C. W. Johnson. The narrow parafacials are mentioned and the "abdomen in males yellow with a wide black dorsal stripe that spreads out posteriorly to cover part of the third and all of the fourth tergum, and with a median ventral longitudinal black stripe also; in the females, black." The forceps are also briefly described.

Townsend (21), in his Manual of Myiology, separates his genus *Cnephalogonia*, of which this species is the type, from related genera on the absence of median marginals on the first segment. This character is purely sexual, these median marginals being present in males of this species and absent in the females of many other species.

The writer prepared his diagrams from a specimen labelled Bar Harbor, Me., 3, viii, 24 C. W. Johnson in the Canadian National Collection, one of the specimens studied by Tothill. Other specimens examined included: two males from Mt. Desert, Me.; two from Aweme, Man.; two from Blackburn, Ont.; 11 males from Low Bush, Ont.; eight females from Low Bush; two from Blackburn and one from Mt. Desert, all in the Canadian National Collection, and one male from Bozeman, Montana.

It seems of value to redescribe this species more fully or at least fill in previous descriptions.

Male: length, 9 to 12 mm. Face and front much less swollen than in any other species of *Gonia*. Parafacials much narrower at the vibrissae than at the aristal bases with two or three uneven rows of setae, which increase in size mesally, and the usual row along the frontal suture. No bare space between other setae and this last row. Genal setae dark, genae about one-third eye height. Occipital pile white. The dorsum of the thorax in certain lights appears to have the usual five wide longitudinal, pollinose lines separated by narrow dull dark lines. In other lights the narrow dull black lines appear pollinose, the wide lines shiny black. The scutellum, varying areas cephalic to the scutellum, and the humeri, yellow. Legs black. *Pulvilli and ungues longer than the last tarsal segment.* Pulvilli white. Ungues slender, curved at the tips and in strong light yellow except at the tips. The abdomen has been well described as to colour by Tothill (quoted above). In most cases it is more orange than yellow. The dorsal and ventral longitudinal stripes are constant and conspicuous; the segmental bases are broadly pollinose. The pollen spreads out especially on the sides of segments 4 to 5 almost the length of the segments. A pair of marginal macrochaetae are present on the second (Townsend's first) segment and a second pair on the third segment. The fourth bears a row of long, strong, marginals, the fifth a row of submarginals with a row of shorter bristles caudad of these.

The forceps are covered on the dorsal side with long, slender, black setae; the dorsal convexity extends down almost to the apex giving them a singular resemblance to those of *longipulvilli* Tothill; however, the extension of the convexity in the last mentioned species is not as great.

Female. Face less swollen than in male, appearing almost flattened. Parafacials wider than in male, considerably narrower at vibrissae than at aristal bases, width at vibrissae about two-thirds greatest eye width.

Thorax as in male. Pulvilli and ungues variable in individuals, from two-thirds to full length of last tarsomere; ungues slender and yellowish basally. Abdomen shining black with only obscured reddening on the sides and venter, no median marginals on the second segment.

Segments pollinose basally, pollen spreading laterally on segments 4 and 5.

Type locality: Westport Factory, Mass.

Distribution: Mass., Conn., Maine, Man., Sask., Mon., Ont.

Type: collection of Boston Society of Natural History.

The North Park specimen referred to by Williston when describing *Gonia exul* was probably of this species.

15. *G. longipulvilli* Tothill

Gonia longipulvilli Tothill (18). (Figs. 15, 15a, 15b)

The drawings were prepared from type No. 789 in the Canadian National Collection. Specimens examined have the pulvilli and ungues of the male longer than the last tarsomere; a narrow or broken dark, longitudinal dorsal line on the abdomen, the remainder of the abdomen laterally and ventrally except for the very base and the apex suffused with an orange yellow colour, no ventral longitudinal dark line being present; forceps with the dorsal convexity extending about three-quarters of their length. The California specimens mentioned by Williston in section (a) following his description of *exul* were probably of this species. This species has been bred from *Agrotis orthogonia* in Saskatchewan.

Type locality: Royal Oak, B.C.

Distribution: the central and western parts of North America from north to south.

Type: in the Canadian National Collection.

16. *G. porca* Will.

1887. *Gonia porca* Williston (24). 1924 *Gonia porca* Williston, Tothill (18), (redescribed). (Figs. 16, 16a)

Drawings were made from a specimen labelled Lillooet, B.C. Aug., 1917, one of the specimens examined by Tothill during his study. As Tothill has pointed out, the yellow hair on the pleurae and the mesonotum make identification of this species simple as do the yellow lobes of the fifth sternite and the shape of the forceps. The depth and chisel-like apex of the forceps is an extreme modification which sets this species very distinctly apart.

Several previously undetermined specimens are in the Canadian National Collection from Jesmond, B.C., 23, vii, 1932, J. K. Jacob, at altitude 7,000

to 7,500 ft., and a series from Lake Louise, Banff, Alta., 22, vii, 1938, G. S. Walley, 8,600 ft.

Type locality: Mt. Hood, Oregon.

Distribution: Oregon, British Columbia, Alberta, Colorado, North Mexico. (Mountainous regions).

Type: ?

17. *G. texensis* Reinhard

1924 *Gonia texensis* Reinhard (12). (Figs. 17, 17a)

The drawings were prepared from paratype No. 791 in the Canadian National Collection, with the same data as the type. This species has been adequately described including the forceps. These latter structures resemble those of *G. porca* Williston in their unusual depth but are greatly modified in shape and vestiture setting this species, too, distinctly apart from all others.

Reinhard says, "In relationship this species is probably nearest to *angusta* Macquart which in Aldrich's catalogue is listed as a synonym of *pallens* Wiedmann, described from Brazil."

(*Gonia angusta* Macquart was described in Diptera Exotique, Paris, Vol. II (3): 51. The author notes that the abdomen is narrower than the thorax, a condition peculiar to *texensis* in our fauna. In 1849 Walker, in Diptera in the British Museum iv: 797, records *angusta* Macq. from Jamaica.)

Type locality: College Station, Texas.

Distribution: Texas.

Type: United States National Museum, Washington.

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sequax group

The above six species form the *sequax* group. The forceps are, in general, deep. Like the group to follow, and unlike the preceding three groups, they are collected in the late summer. The last two species, at least, are widely divergent from the close relationships so evident among other species and are similarly of limited habitat. It seems probable that transition forms between these and other types may exist.

The last or *fissiforceps* group consists of three species so closely related as to cause some doubt as to their specific rank and yet so widely separated from all the other species as to stand alone. Their habitat only they have in common with *porca* Williston, and their occurrence in late summer in common with the *sequax* group.

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18. *G. fissiforceps* Tothill

1924 *Gonia fissiforceps* Tothill (18). (Figs. 18, 18a, 18b)

This large, dark, mountain species is easily recognizable from the diagrams given here and from the description by Tothill. It might be added that the

palpi vary from yellow to black, the pulvilli are white and almost as long as the last tarsomere in males. The "escalloped" inner edges of the lobes of the fifth sternite are characteristic of this and the following two species.

The new species *tenuiforceps* is being separated from this species on the shape of the forceps alone. One of Tothill's paratypes labelled Banff, Alta., N. B. Sanson, belongs to the new species.

Type locality: Lillooet, B.C.

Distribution: Banff (Alberta), Colorado, Washington, California, Ontario.

Type: No. 782, Canadian National Collection.

19. *G. tenuiforceps* new species. (Fig. 19, 19a)

Male: length, 10 to 11 mm. Shiny black species. Face and front light yellow, with a silvery sheen in some lights; the front above with a waxy appearance on the sides; parafacials not narrowing greatly from opposite aristal bases to oral margin, at the narrowest point slightly less than or equal to the greatest eye width, with a double or triple irregular row of slender black setae along the inner edge of the eye. The usual row of setae along the edge of the frontal suture irregular in extent and in size of bristles; genae about one-half the eye height with a few scattered black setae. Antennae black with a greyish tinge in some lights, second segment reddish or yellowish apically with short, dark, dorsal setae; third segment five to seven times as long as the second. Pile on the occiput white. Palpi yellow through various degrees of infuscation to black. Thorax shiny black, sometimes with powdering of white pollen, which may be distinctly divided into five lines by very narrow shiny areas; scutellum yellow; legs black, femora and tibiae (each or both) in strong light may show yellow areas. Pulvilli and ungues (about) as long as last tarsomere. Pulvilli white. Ungues slender, and, in strong light, yellow at the base and tip.

Abdomen shining black, segments 3, 4, and 5, sometimes narrowly, evenly white pollinose basally. Rarely with indications of yellowish red colouring on the sides of segments 2, 3, and 4. The lobes of the fifth sternite are definitely excised laterally on the inside, giving a "scalloped" effect (Fig. 18b), as in *fissiforceps* and *yukonensis*.

Forceps long, resembling those of *fissiforceps* but having much less depth medially, dorsal convexity extending less than one-third of their length, apex turned ventrally (forward) to form a hook, in dorsal view the median apical cleft extends beyond the middle.

Type: Male, Hopedale, Labrador, 21, vii, 1926, W. W. Perret,—No. 5035 Canadian National Collection.

Paratypes: As above; 23, vii, 1926,—17, vii, 1926,—25, vii, 1924,—10, vii, 1926,—22, vii, 1926. Two females 18, vii, 1926. One paratype of *fissiforceps* Tothill, Banff, Alta., ?, N. B. Sanson.

20. *G. yukonensis* Tothill

Gonia yukonensis Tothill (18). (Figs. 20, 20a, 20b)

Drawings were prepared from the type, No. 786, Canadian National Collection.

Tothill records a paratype of this species from Tennessee Pass, Colo. (in the United States National Museum). His description of the median apical cleft of the forceps "extending about one third distance to base" proved a little inaccurate, when the forceps had been treated in potash. The cleft is seen to extend almost half the distance to the base. The similar habitat and the shape of lobes of the fifth sternite (not mentioned by Tothill) suggest a very close relationship with *fissiforceps*. The forceps, however, have the dorsal convexity extending further down the length of these structures (about one-third the distance to the apex) than in any *fissiforceps* observed. For the present it is thought best to maintain this name but there is considerable doubt in the writer's mind as to the specific rank of this specimen.

Type locality: Yukon Territory.

Distribution: Yukon, Colorado.

Type: No. 786, Canadian National Collection.

21. *G. carinata* Tothill

1924. *Gonia carinata* Tothill (18).

There being no known specimens of this species besides the holotype, which was not available to the writer, diagrams of this species could not be included. The following is Tothill's original description:

"Parafacials at narrowest point about three times the length of the second antennal segment and only slightly wider at the base of the antennae than at vibrissae. Genal hairs fuscous. Third antennal segment black. Occipital pile white. Pleura without golden pile. First vein without bristles; third vein with a few small bristles at base. Abdomen reddish yellow except for a dorsal and a ventral narrow black stripe and except for the last tergum which is black.

"The forceps of genitalia remarkable for the long median carina that is as deep as the forceps are thick. Otherwise the forceps are long and narrow with the notch confined to the apical fourth, and straight in ventral profile."

Holotype: Male; from Salt Lake, Utah; in United States National Museum.

Tothill merely listed the species in the order in which they occurred in his key, though he suggested a western origin and eastward spread of this genus. The information at hand seems inadequate to support or deny this hypothesis. However, the tentative order of the species in this paper is based on distribution, time of occurrence, and especially similarities in the structure of the forceps.

Frontosa is our most widely spread species, *fuscicollis* is undoubtedly closely related to it, and *sagax* though unique in its antennal colour resembles these two in the structure of the forceps. In the *aldrichi* group the forceps maintain to some extent their curved ventral edge and dorsal apical convexity but have become elongated and flattened. In the *breviforceps* group the ventral edge of the forceps has become straight and an increase in depth is evident. Moreover, the range appears more restricted. The *sequax* group includes species from widely separated areas. They are separated from each other more distinctly than are the members of other groups. In all these species the forceps have increased markedly in depth. The *fissiforceps* group includes, as previously noted, extremely closely related species, but as a group shows no close affinities to any other species. The habitat of the last group is limited to mountainous areas. It may thus overlap the habitats of *porca* and possibly *distincta*. The shape of the lobes of the fifth sternite and the shape of the forceps set the *fissiforceps* group apart.

Since *carinata* could not be examined nor even the date of capture ascertained it could not be fitted into this arrangement and is consequently listed last.

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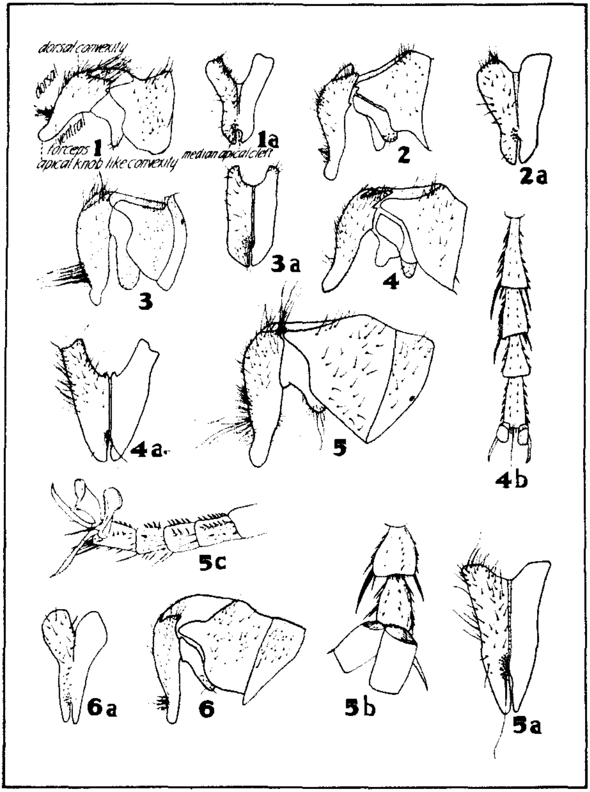
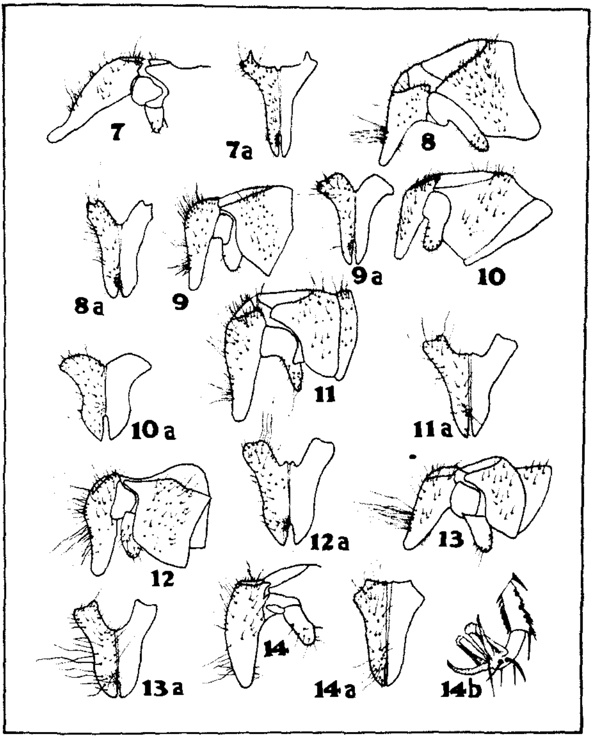
Explanation of Figures

Figs. 1 to 22 have been prepared in so far as possible from type material. In many cases the sclerites behind the forceps were broken or twisted and were drawn as observed. The exact position of the structures when drawn was that in which the forceps were most distinctive, and it varies slightly with different specimens. In all cases it is the contour of the forceps and not the exact shape of the other sclerites that is specific. The density, distribution, and length of setal vestiture varies and is often affected by the way material has been handled. It has, however, been indicated so far as possible. The linear magnification is in all cases approximately 30.



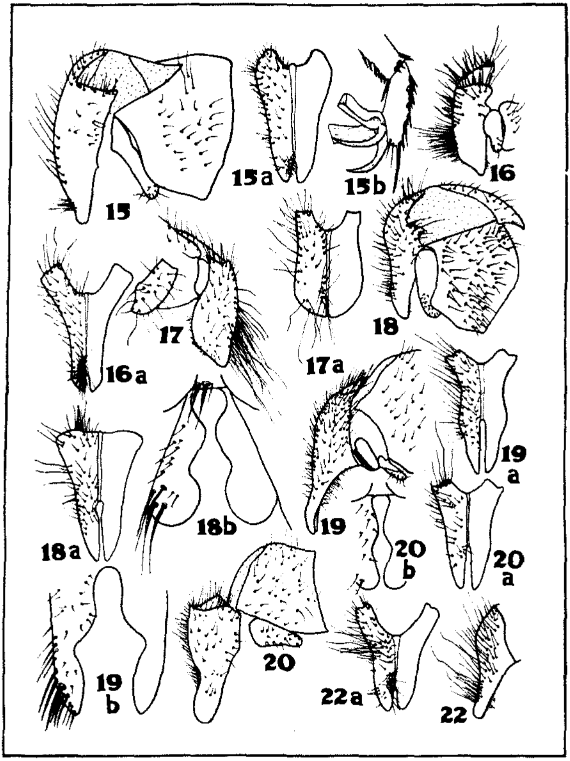
Figs. 1 to 6a.

1. *Lateral view of the forceps of G. frontosa Say.* 1a. *Dorsal view of the forceps of G. frontosa Say.* 2. *Lateral view of the forceps of G. sagax Townsend.* 2a. *Dorsal view of the forceps of G. sagax Townsend.* 3. *Lateral view of the forceps of G. fuscicollis Tothill.* 3a. *Dorsal view of the forceps of G. fuscicollis Tothill.* 4. *Lateral view of the forceps of G. aldrichi Tothill.* 4a. *Dorsal view of the forceps of G. aldrichi Tothill.* 4b. *Anterior tarsus, claws and ungues of G. aldrichi Tothill.* 5. *Lateral view of forceps of G. grandipulvilli new species.* 5a. *Dorsal view of the forceps of G. grandipulvilli new species.* 5b. *Dorsal view of last two tarsomeres, pulvilli and ungues of the prothoracic leg of G. grandipulvilli, new species.* 5c. *Lateral view of the same.* 6. *Lateral view of the forceps of G. longiforceps Tothill.* 6a. *Dorsal view of the forceps of G. longiforceps Tothill.*



Figs. 7 to 14b.

7. *Lateral view of the forceps of G. discalis new species.* 7a. *Dorsal view of the forceps of G. discalis new species.* 8. *Lateral view of the forceps of G. breviforceps Tothill.* 8a. *Dorsal view of the forceps of G. breviforceps Tothill.* 9. *Lateral view of the forceps of G. albagenae new species.* 9a. *Dorsal view of the forceps of G. albagenae new species.* 10. *Lateral view of the forceps of G. setigera Tothill.* 10a. *Dorsal view of the forceps of G. setigera Tothill.* 11. *Lateral view of the forceps of G. turgida Coquillet* 11a. *Dorsal view of the forceps of G. turgida Coquillet.* 12. *Lateral view of the forceps of G. sequax Williston.* 12a. *Dorsal view of the forceps of G. sequax Williston.* 13. *Lateral view of the forceps of G. senilis Williston.* 13a. *Dorsal view of the forceps of G. senilis Williston.* 14. *Lateral view of the forceps of G. distincta Smith.* 14a. *Dorsal view of the forceps of G. distincta Smith.* 14b. *Last tarsomere, pulvilli and ungues of the prothoracic leg of G. distincta Smith.*



Figs. 15 to 22a.

15. *Lateral view of forceps of G. longipulvilli Tothill (sclerites of segment 9 badly twisted).* 15a. *Dorsal view of forceps of G. longipulvilli Tothill.* 15b. *Last tarsomere, pulvilli and ungues of the prothoracic leg of G. longipulvilli Tothill.* 16. *Lateral view of the forceps of G. porca Williston.* 16a. *Dorsal view of the forceps of G. porca Williston.* 17. *Lateral view of the forceps of G. texensis Reinhardt (sclerites of segment 9 badly broken).* 17a. *Dorsal view of the forceps of G. texensis Reinhardt.* 18. *Lateral view of the forceps of G. fissiforceps Tothill (sclerites of segment 9 badly broken).* 18a. *Dorsal view of the forceps of G. fissiforceps Tothill.* 18b. *Lobes of the fifth sternite of G. fissiforceps Tothill.* 19. *Lateral view of the forceps of G. tenuiforceps new species.* 19a. *Dorsal view of the forceps of G. tenuiforceps new species.* 19b. *Lobes of the fifth sternite of G. tenuiforceps new species.* 20. *Lateral view of the forceps of G. yukonensis Tothill.* 20a. *Dorsal view of the forceps of G. yukonensis Tothill.* 20b. *Lobes of the fifth sternite of G. yukonensis Tothill.* 22. *Lateral view of the forceps of G. capitata (DeGeer)?* 22a. *Dorsal view of the forceps of G. capitata (DeGeer)?*

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FROZEN STORAGE OF POULTRY

IV. FURTHER OBSERVATIONS ON SURFACE DRYING AND PEROXIDE OXYGEN FORMATION[1]

BY W. H. COOK[2] AND W. H. WHITE[2]

Abstract

A package constructed from moisture resistant material, capable of being ventilated during chilled storage, and sealed to prevent surface drying during frozen storage, is described. Results are presented to demonstrate the ability of this package to maintain the desired humidity conditions. Jacketing a room to separate the cooling coils from the space occupied by the product does not prevent surface drying of boxed goods, presumably because of the absorption of moisture by the boxes. Delays between slaughter and freezing accelerate the development of rancidity in the fat of poultry during subsequent frozen storage, as indicated by the formation of peroxide oxygen. The free fatty acid content is not seriously affected unless the conditions prior to freezing enhance microbial development.

Introduction

It has been shown in earlier papers of this series (1, 2, 3) that surface drying, causing a loss of bloom and development of freezer burn, was the first type of deterioration to occur in poultry during frozen storage. It was also found that conditions favouring surface drying also promoted the development of rancidity in the fat. These results suggested the present studies on methods of packaging for minimizing surface drying, and the effect of delays between slaughter and freezing on the development of rancidity.

The results of previous investigations showed that surface drying could be minimized by lining the boxes with reasonably moisture-resistant stocks, such as waxed paper, provided the folds and joints were adequately sealed. Since adequate sealing of the liners used in wooden poultry boxes is commercially impracticable, other types of packages were studied. One disadvantage of a sealed package is that it maintains a high humidity within the package under all conditions, and during chilled (unfrozen) storage such a condition enhances microbial growth. As a certain proportion of market poultry is held in the chilled state for immediate domestic consumption there is an obvious need for a package that can be readily ventilated or sealed in accordance with trade requirements.

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Packaging Experiments

Description of Packages

Initial experiments were undertaken with several standard types of corrugated cartons. Those having telescoping covers proved to be best from the standpoint of packing, storing, and sealing. Commercial trials indicated that the strength of such a package was generally satisfactory for a six-bird size. Standard 12-bird packages were also used experimentally, and the use of heavy corrugated stocks and containers of suitable design would appear to make this size entirely practicable for net weights up to 50 or 60 lb.

Cartons of this type must be rendered moisture-resistant. This was accomplished by various methods, including the application of wax or aluminium foil on one or both sides of the carton. Although the foil has many desirable features, the results of these preliminary tests indicated that the application of a sufficiently heavy coating of wax to produce a glossy surface on the inside was adequate for protecting the product. For commercial use the application of wax to the outer surface would also seem desirable to protect the package against condensate and other contact with moisture. All subsequent tests were conducted with both inside and outside surfaces waxed. No liners were used.

Although the ordinary type of full-telescoping carton was easier to seal than the liners in wooden boxes, it was still found difficult to obtain an effective seal in routine practice. This led to the design of the half-telescoping carton shown in Fig. 1, which proved to be comparatively easy to seal, and also facilitated storage of the product in either a "ventilated" or sealed condition. In this package the full size inner tray fits into another tray of half the height, and is covered by a similar half-height tray. These two cover members meet along the medial line of the container, and can be effectively sealed with a moisture-resistant adhesive tape. This construction also provides a double bottom to strengthen the package. If necessary, the carton can be strapped or wired before storage or transport.

The necessary ventilation during chilled storage was obtained by providing a hole in each end of the inner tray just above the joint of the two outer members. By suitably stamping the telescoping cover opposite these holes it was possible to provide a flap that could be broken open to expose the holes when ventilation was desired, or sealed beneath the tape to provide a moisture-tight package. Previous tests demonstrated that a relative humidity range of 85 to 90% was obtained during chilled storage when the openings in the inner tray were ½, ¾, and 1 in. for boxes designed to contain about 25, 40, and 60 lb. of poultry respectively. Liners were not used in these packages since they were unnecessary and might obstruct the openings.

Results

Semi-commercial scale tests were conducted with the new design of package in both chilled and frozen storage. The relative humidity inside the package was taken as the criterion of proper ventilation in the chilled state, while the

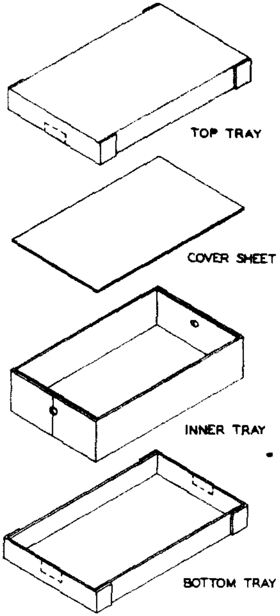

FIG. 1. *N.R.C. design of corrugated carton used to facilitate ventilation or sealing.*

proportion of the surface area affected by freezer burn after 27 to 32 weeks' storage at $-12°$ C. was used as a measure of its value for protecting the frozen product against drying.

The chilled storage tests were of 30 days duration at 0° C. During this period two relative humidity measurements were made on each of the experimental boxes using the special hair hygrometer referred to in earlier work (1). Observations were made on both ventilated and sealed packages of the type described earlier, and also on a few of the older types of containers. The results in Table I show that the ventilated N.R.C. type maintained relative humidities between 85 and 90%, which is considered satisfactory. This package in the sealed form, and all the other containers tested, maintained

TABLE I

RELATIVE HUMIDITY IN POULTRY BOXES DURING 30 DAYS' STORAGE AT 0° C.

Type of box	No. boxes in test	Range of size (net wt. poultry, lb.)	Relative humidity inside boxes during storage		
			Max.	Min.	Av.
N.R.C.—ventilated	5	19–75	90	84	86
N.R.C.—sealed	5	22–74	100	98	99
Ordinary telescoping carton—unsealed	1	28	—	—	98
Wooden box with unsealed moisture-resistant liner	2	42–50	98	100	99

humidities approaching saturation, which is clearly too high for satisfactory storage in the chilled state.

The extent of surface drying in the various containers stored in the frozen state is evident from the results presented in Table II. The average value of 2% indicates that the new containers give adequate protection from surface drying. For the most part the small freezer-burnt areas occurred only on certain birds that had been forced in close contact with lightly waxed regions of the cartons. Experienced inspectors examining these boxes reported no evident deterioration, and classed the bloom as "good" or "excellent".

Although only a limited number of other types of boxes were available for comparison, the results are in complete agreement with those obtained in earlier preliminary experiments. An ordinary waxed but unsealed telescoping

TABLE II

SURFACE DRYING DURING STORAGE IN FROZEN STATE

Type of box	No. boxes in test	Storage conditions	Proportion of surface area affected by drying			Remarks
			Max., %	Min., %	Av., %	
N.R.C.—sealed	8	27 weeks at −12° C. 65% R.H.	8	1	2	
N.R.C.—sealed	11	32 weeks at −12° C. 65% R.H.	3	1	2	Figures exclude one damaged box showing 10% F.B.
Ordinary telescoping carton—unsealed	2	32 weeks at −12° C. 65% R.H.	30	3	14	
Wooden box with unsealed moisture-resistant liner	1	32 weeks at −12° C. 65% R.H.	—	—	14	
Wooden box with sealed moisture-resistant liner	2	27 weeks at −12° C. 65% R.H.	3	1	2	

carton, and the usual wooden box with unsealed liner both showed about 14% of the area of the product affected by freezer burn. Inspectors reported deterioration in these boxes. Sealing the waxed paper liner in the wooden boxes reduced the affected area to 2%, in agreement with earlier results (1).

One of the recent developments for preventing drying and shrinkage of individually wrapped birds during storage is the use of thin transparent latex bags. Ten birds were sealed in these bags and exposed, without further protection, for a period of 43 weeks to the conditions described earlier. At the end of the storage period there was no evidence of drying, and the loss in weight was less than 1%.

Experiments in Jacketed Spaces

Primarily, a moisture-resistant packaging provides a vapour barrier between the product and the cooling coils, the regions of maximum and minimum vapour pressure respectively. The introduction of this vapour barrier in the same relative location, but as part of the room rather than as part of the package, might prove equally effective and less costly in the prevention of drying. The use of a jacketed room, as suggested by Huntsman (4), with the cooling coils placed between the jacket and the insulated wall, appears to meet these requirements. Although a cold store of this type would doubtless reduce desiccation, it might not be as effective as moisture-resistant packaging, since there is some evidence (5) that packages such as wooden boxes may themselves absorb considerable moisture from the air at relative humidities approaching saturation. In these circumstances the cooling coils may not be the only agency responsible for drying, and consequently their isolation from the space occupied by the product may be only partially effective in reducing desiccation.

This possibility was examined by placing two boxes of poultry in each of two gas-tight steel tanks to represent jacketed spaces. These were of sufficient size to contain two boxes of poultry in $\frac{2}{3}$ to $\frac{3}{4}$ of their volume. Approximately 20 lb. of ice was placed in the bottom of one of the tanks, in order to provide a source of water vapour, other than the product, for the maintenance of a high relative humidity. The other tank contained only the boxed product. In all instances the poultry was packed in wooden boxes with moisture-resistant unsealed liners. The tanks were stored at a temperature of from -12 to $-15°$ C. for a period of 87 weeks before being opened for examination. This prolonged storage period was used to exaggerate any surface desiccation that might have occurred.

Results showing the condition of the poultry in the two tanks at the end of the storage period are given in Table III. Serious deterioration of the product had occurred in the tank without ice, while that in the tank containing ice did not show marked injury. Although this product was stored for an excessively long period, the results nevertheless demonstrate that the absorption of moisture by the package may cause serious surface desiccation. In these circumstances it is evident that goods packed in containers capable

TABLE III

SURFACE DRYING FOLLOWING 87 WEEKS' STORAGE AT −12 TO −15° C. IN GAS-TIGHT TANKS WITH AND WITHOUT ICE

Storage conditions	Surface area affected by desiccation, %	Bloom
In tank without ice	20–25	Poor
	10–15	Poor
In tank with ice	0–5	Good
	0–5	Excellent

of absorbing moisture cannot be stored successfully in a jacketed room unless a high humidity is maintained by some agency other than the stored product.

Formation of Peroxide Oxygen and Free Fatty Acids

The results of a previous investigation on the frozen storage of poultry showed that the fat was relatively resistant to oxidative and hydrolytic changes (3). Although surface desiccation accelerated peroxide oxygen formation, it was found that, even under conditions favouring severe drying, the peroxide oxygen content seldom exceeded 8.0 ml. of 0.002 *N* sodium thiosulphate after a storage period of 25 months at −13.5° C. It was concluded that the extent of the decomposition of the fat should seldom effect serious deterioration in the flavour of poultry which was promptly precooled and stored at suitable temperatures in the frozen state for normal storage periods.

In commercial practice, delays in cooling or freezing may unavoidably occur, and if sufficiently prolonged may result in a considerable acceleration of the decomposition of the fat. Although such changes may not be evident immediately because of the nature of the induction period characteristic of the development of rancidity, the fat may become rancid quite rapidly during a subsequent period of frozen storage. The material available from the packaging experiments described previously permitted some preliminary observations on this problem.

Peroxide oxygen and free fatty acid determinations were made by methods previously described (3), on the subcutaneous and skin fat of one or two birds taken at random from each of a number of the boxes at the end of the period of frozen storage. This material represented poultry that had been precooled and packed in a commercial plant, and which had been held at temperatures of approximately 0° C. for periods of one week and five to six weeks between slaughter and freezing. Since none of the boxes from which the samples were taken suffered evident deterioration from drying, the accelerating action of surface desiccation on peroxide oxygen formation was excluded.

The peroxide oxygen and free fatty acid contents of the fat and the corresponding storage conditions are shown in Table IV. Poultry held for one week at 0° C., followed by frozen storage at −12° C. for 32 weeks, yielded peroxide oxygen values approximately twice as large as those previously obtained for poultry stored for much longer periods (3). This suggests that delays before precooling or freezing were responsible for the greater deterioration. The free fatty acid contents for this group of poultry were low and approximately normal.

TABLE IV

PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF POULTRY FAT FOLLOWING VARIOUS STORAGE TREATMENTS

Storage conditions	Peroxide oxygen (as ml. 0.002*N* $Na_2S_2O_3$ per gm.)	Free fatty acid as % oleic acid
Precooled commercially, stored one week at 0° C., and 32 weeks at −12° C.	4.0	0.57
	2.7	0.59
	3.0	0.67
	0.8	0.54
	4.9	0.65
	1.7	0.53
Precooled commercially, stored 5 to 6 weeks at 0° C., and 27 weeks at −12° C.	6.3	5.3
	8.8	19.9
	11.1	15.2
	5.1	31.4
	18.4	12.9
Precooled commercially, frozen and stored in a gas-tight tank containing ice for 87 weeks at −12 to −15° C.	9.3	0.95
	6.3	0.60
	6.4	0.88
	11.7	0.89

The next series of samples studied were obtained for poultry stored for five to six weeks at 0° C., followed by storage for 27 weeks at −12° C. Under these conditions the peroxide oxygen values had increased to levels at which the fat would be considered rancid. In addition the free fatty acid content was high, and would undoubtedly exert a deleterious effect on flavour. The excess free fatty acid formation may be attributable to excessive microbial activity during storage at 0° C. These results show conclusively that the product should be precooled and frozen promptly after slaughter in proper storage practice.

It is evident that there is considerable variation in the peroxide content of the fat of birds treated similarly. This indicates a difference in the susceptibility of the fat of different birds to oxidation, and is in agreement with previous findings (3). Doubtless the breeding and feeding of the poultry affects the susceptibility of the fat to oxidation.

The last section of Table IV gives the results of the analysis of the fat of two birds taken from each of the two boxes stored in the gas-tight tank con-

taining ice (see Table III). The peroxide oxygen values were generally higher than those observed previously (3). This may be due to greater delays in precooling and freezing the present material, or to a greater susceptibility of the fats of these birds to oxidation. Nevertheless it is evident that poultry fat may become slightly or definitely rancid under storage conditions that prevent surface drying. Rancidity development is therefore one of the factors limiting the storage life of poultry stored at −12° C. even when surface drying is prevented. These birds were stored for an excessive period, however, and under commercial storage practice, surface drying, which is directly detrimental to quality, and indirectly accelerates oxidative changes, is likely to be the primary factor limiting the storage life.

Acknowledgments

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

VIII. COMPARISONS OF THE LETHAL EFFECTS OF SOME CHEMICALS CONTAINING SULPHUR ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

By I. W. Parnell[2]

Abstract

The effect on the free-living stages of sclerostomes of 14 common chemicals containing sulphur is discussed. Potassium xanthogenate in weak solution will sterilize about 160 times its weight of fresh faeces; in a strong solution, less than a quarter of the weight. Carbon disulphide will sterilize only about 32 times its weight under the conditions imposed by this technique. In solutions of medium strength the sulphates of zinc will sterilize 32 times, and those of iron (ferrous), copper, and manganese, 13 times their weight; lesser quantities, however, might be effective in practice, because the larvae from cultures so treated die comparatively rapidly after reaching the third stage. Dry ferric sulphate will sterilize 20 times its weight. Sodium sulphide, sodium sulphite, and sodium sulphate are all so ineffective that only when applied dry or as strong solutions are they lethal; they sterilized from eight to five times their weight. Magnesium sulphate will sterilize only four times and zinc sulphide twice its weight of fresh faeces. Ferrous sulphide and flowers of sulphur do not appear to have any lethal value, but the latter may, by controlling antagonistic factors, increase the chances of survival of the larvae.

Introduction

This paper reports on the lethal properties, against the free-living stages of Sclerostomes in fresh faeces, of some chemicals containing sulphur.

Potassium xanthogenate and carbon disulphide were tested because they have, under some conditions, proved effective against plant nematodes. Cupric sulphate was tested because it has for many years been suggested for treating pastures to kill nematode eggs and larvae, is used on pastures to kill snails, and is the standard chemical to use as an anthelmintic against some adult bursate nematodes. Flowers of sulphur was tested because it is the standard lethal agent against certain pests. Zinc sulphate, ferric sulphate, ferrous sulphate, manganous sulphate, sodium sulphide, sodium sulphite, sodium sulphate, magnesium sulphate, and ferrous sulphide were selected for testing because they are salts of the more important minor elements necessary for plant growth and for comparison with the chlorine salts of the

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same metals (to be discussed in the next paper), and also because it has been shown that sulphur and chlorine in some chemicals can exert important influences on the sheath and on the exsheathment of larvae when free of faeces (21, 22, 23). For instance, some sulphides may alter the permeability of the sheath and might be of considerable importance in bringing the lethal chemicals into contact with the larvae. It has been shown that some sulphur compounds are more effective than analogous oxygen compounds against mosquito larvae, with the exception of diphenylene oxide (3). Ammonium sulphate (32), potassium sulphate (33), and nicotine sulphate (34) have been discussed in previous papers.

Copper sulphate has been tested relatively frequently against the free-living stages of bursate nematodes, especially in Germany. Some of the more recent papers include data comparable with those in this paper. In 1930 it was reported that a 1% solution rapidly made third-stage Sclerostome larvae motionless, and that even a 0.02% solution had this effect in a few hours; furthermore, on being removed to water they did not recover (29), a characteristic effect of copper sulphate, which had been previously noted (1). However, it was shown, about the same time, that against Sclerostome eggs and third-stage larvae in faeces, 1 and 2% solutions were not satisfactory (38). In 1934 it was reported that 1, 0.5, and 0.1% solutions were lethal to third-stage Sclerostome larvae in 15 to 30 hr., but that a 1% solution did not prevent the eggs maturing to the third stage, which a 2% solution did prevent. It was also reported that when a 0.1% solution was used on grass, about 50% of the larvae survived, but only 3 to 8% survived when a 1% solution was applied (12). It has since been stated that the addition of copper sulphate to kainit adds to the efficiency of the latter, and that under laboratory conditions a 4 : 1 kainit: copper sulphate mixture at the rate of about 300 lb. per acre killed all the eggs, but that out of doors the results were less promising (24).

Copper sulphate has also been tested against the eggs and larvae of *Stephanurus dentatus*. A 1 : 1,000 solution killed both free-feeding and infective larvae in 24 hr., and stronger solutions were more rapidly lethal. However, against infective larvae in mud, a 1 : 10 solution took 72 hr. to be effective when applied at the rate of 10 gal. per 100 sq. yd. (39), whereas a 1% solution at the rate of approximately 4 gal. per 100 sq. yd., although frequently applied, was not effective against either *Stephanurus* or *Oesophagostomum* larvae (40). A 12½% copper sulphate solution has been tested for 30 min. against *Anguillulina dipsaci* in sweet potatoes (20), and for two hours, as a 2, 1, and 0.5 solution against *Tylenchus (=Anguillulina) dipsaci*; in two hours the 2% solution was lethal, but not the weaker solutions (28). Under similar conditions, 2% solutions of ferrous sulphate, sodium sulphate, and sodium sulphite were not lethal (28). Copper sulphate and sodium sulphite were not lethal (28). Copper sulphate has also been reported as useless against *Heterodera schachtii* (4).

Copper sulphate applied to manure at the rate of one-quarter pound and one pound in one gallon (American) of water per cubic foot of manure killed about 60% of fly larvae; iron sulphate was not effective (6). Iron sulphate has been tested less frequently than copper sulphate against nematodes. As a 1% solution it did not prevent hookworm eggs from hatching, but killed the free-feeding larvae (35). At the rate of 1680 lb. per acre, ferrous sulphate did not control the cysts of the *Heterodera schachtii* (10, 11), but in other small scale experiments ferrous sulphate did control potato sickness caused by *H. schachtii*, although less rapidly than did ferric chloride (18).

A few other sulphur salts, such as magnesium sulphate, have been tested against various pests. Magnesium sulphate was reported to kill wireworm (*Agriotes mancus*) larvae in soil (17), but not to kill fly larvae in manure (7). Sodium sulphide as a 0.6% solution was stated to be effective against *Tylenchus dipsaci* in two hours (27).

Sulphur has frequently been tested for the control of plant nematodes; the results, like those with so many other chemicals, are difficult to compare and are contradictory. It has been stated that 400 to 600 lb. of powdered sulphur per acre, prevented root-knot of sweet potatoes, and that even 200 lb. was beneficial (36); 250 to 500 lb. per acre has also been recommended against root-knot (42). Sulphur, at the rate of one pound per bushel of manure, was effective against fly larvae (5). Mixed with formaldehyde, it was reported as being more effective than lime against *Heterodera marioni* (43), but alone was useless against *H. marioni* (2), and against *H. schachtii* (19, 25), even with dressings of 500 to 1000 lb. per acre (37). In greenhouses, although applied at the rate of 2600 lb. per acre, sulphur gave no benefit against root-knot (26).

Carbon disulphide and potassium xanthogenate (which on decomposition releases carbon disulphide believed to be the cause of the latter's toxicity (30)) have also frequently been tested against plant nematodes. Again the reports show how varied the results have been. In pot experiments, carbon disulphide was stated to be of no value (4). At a rate of 220 gal. (American) per acre, carbon disulphide was also reported to be of no benefit in greenhouses (26), and at 30 gal. did not affect materially the degree of infection of potatoes with nematode disease (9). It was, however, stated to be superior to calcium cyanide, calcium cyanamide, and quicklime (8), but less effective than formaldehyde (43). Applied as an emulsion, containing 68% carbon disulphide, further diluted with 50 parts of water to one of emulsion, one gal. (American) per sq. ft. was reported to be effective against *H. marioni* (14). At the rate of four pounds per 100 cu. ft. of soil, when the temperature was 77° F., carbon disulphide was completely effective against *Rhabditis lambdiensis*, and two and a half to three pounds per cubic foot killed most of those mushroom parasites (16).

In pots, carbon disulphide also reduced the number of cysts on potatoes (25). At the rate of 750 lb. per acre it decreased nematodes by 48% (13), while 13.0, 20.0, and 30.0 cc. reduced *H. marioni* in 12 kg. of sandy soil to

6.4, 5.2, and 0.61% respectively (15). In practice, it was reported to give satisfactory results (42), especially against nematodes on trees and shrubs (41).

Potassium xanthogenate has been shown to be more effective against *H. radicicola* than against *H. schachtii;* larvae of the former are killed by 0.35 gm. per 300 cc. (=2750 lb. per acre foot) in light soil (30). The vapours of potassium xanthogenate were reported to penetrate soil and paralyse larvae in three days, and in small-scale experiments, at the rate of 140 lb. per acre, to have controlled potato sickness (18).

Methods

Full descriptions of the technique used to obtain the data on the lethal values of chemicals against the free-living stages of Sclerostomes have been given in earlier papers (31, 33). The results obtained with the chemicals discussed in this paper were all obtained by the same methods. Cultures of 40 gm. of *fresh* (less than four or five hours old) horse faeces, were treated with the chemical to be tested, and left to develop in a dark constant temperture room at 26° C. ± 3° C. for several days or weeks; the larvae were then extracted in funnels, from which they were drawn on the tenth day. This technique probably gives a very exacting test of the chemical, since the conditions are very suitable for rapid development of the larvae. However, some larvae may escape contact poisons by crawling up the walls of the glass container, or gases by remaining in small lumps of faeces. When the larvae reach the infective stage and then die, it is possible to recover only those on the outside of the culture or on the walls of the container.

Exact data are not available, but the sediment visible in the tap water used during the extraction process suggested that the quantity of impurities increased during the latter part of this work. These impurities occasionally amounted to about 0.01 gm. per 500 cc., chiefly consisting of iron carbonate.

The 9-in. extraction funnels held about 1600 cc., and approximately the same quantity is poured off and replaced during the extraction process. However, it is probable that the concentration of impurities was insufficient to affect the larvae, either by making them exsheath or otherwise injuring them.

Results

Potassium Xanthogenate

Potassium xanthogenate was tested dry and in solution; the results obtained are illustrated in Fig. 1.

It was tested dry in quantities ranging from 0.25 to 20% of the faeces by weight. The addition of 0.2 to 0.5 gm. considerably reduced the number of larvae. However, from some of the cultures to which 1.0 gm. or more was added, a few larvae, many dead, were recovered; from one of the cultures treated with 0.75 gm., equivalent to 1.87%, a few larvae were obtained; the other two cultures were sterilized.

A 1 : 2 aqueous solution was applied in quantities of 0.25 to 15.0 cc.; the addition of 1.5 to 2.0 cc. reduced the number of larvae recovered to a

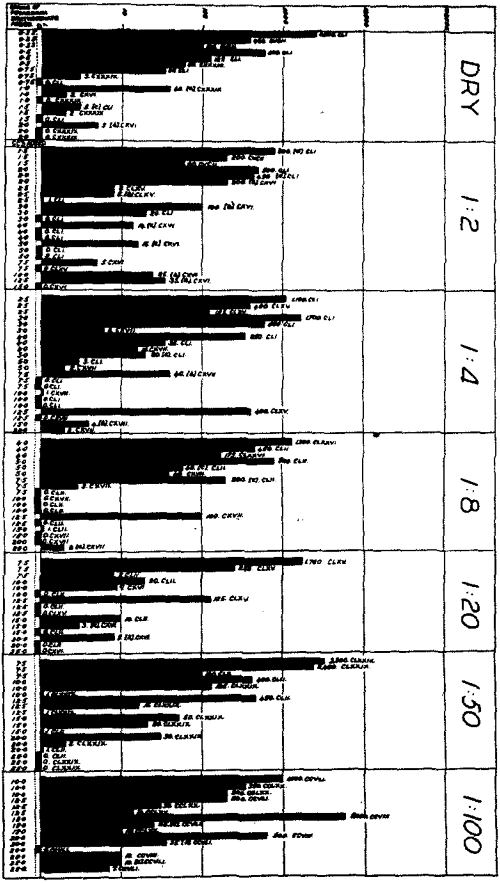

FIG. 1.

few hundred, and 2.5 cc. almost sterilized the cultures. However, from some of the cultures treated with 3.0 cc., and more, a few larvae, many of which were dead, were recovered. In 2.5 cc. of a 1 : 2 solution, there is almost 1.0 gm. of potassium xanthogenate, or 2.5% of the weight of the faeces.

The results obtained with a 1 : 4 solution, which was added in quantities of 0.5 to 20.0 cc., were also extremely irregular, but they suggest that 5.0 cc. will cause sterilization, although 400 larvae were recovered from one culture treated with 12.5 cc. In 5.0 cc. of solution of this strength there is slightly more than 1.0 gm., equivalent to 2.75% of the weight of faeces.

All the cultures receiving with 3.0 cc., or less, of a 1 : 8 solution yielded thousands of larvae. From two cultures treated with 5.0 cc. only 60 and 40 larvae were obtained; two of three cultures were sterilized by 7.5 cc. and all three cultures by 10.0 cc. One hundred larvae were recovered from one culture treated with 12.5 cc. In 10.0 cc. of a 1 : 8 solution there is also slightly more than 1.1 gm.

As a 1 : 20 solution, 2.0 cc. was the smallest quantity added. The results were again irregular; one culture was almost sterilized by 7.5 cc., 10.0 cc. was almost effective in three cultures, but 125 larvae were recovered from one receiving 12.5 cc. In 10.0 cc. of a 1 : 20 aqueous solution there is only slightly less than 0.5 gm., or 1.25% of the weight of the faeces in the cultures.

With one exception, all the cultures treated with not more than 7.5 cc. of a 1 : 50 aqueous solution contained thousands of active Sclerostome larvae. The addition of 10.0 cc., or more, considerably reduced the number of larvae in all cases. If the results, which were again irregular, are averaged, they suggest about 15.0 cc., containing 0.3 gm., equivalent to only 0.75%, as the approximate quantity necessary to sterilize the faeces.

A 1 : 100 aqueous solution was added in quantities ranging from 3.0 to 25.0 cc. All cultures receiving 5.0 cc., or less, yielded many thousand larvae. In two cultures, 7.5 cc. reduced the larvae to 700 and 550; in two, 10.0 cc., reduced them to 350 and 200. Those treated with 12.5 cc. yielded only 200, 30 and 14, but from one culture with 15.0 cc., 5600 larvae were isolated, and 600 from a culture treated with 20.0 cc. The other cultures receiving 15.0 and 20.0 cc. were practically sterilized, as were three to which had been added 25.0 cc., which contains about 0.25 gm., or 0.63% of the weight of the faeces.

Potassium xanthogenate was also tested as a 1 : 200, as a 1 : 300, and as a 1 : 400 aqueous solution; the results obtained are not illustrated. The 1 : 200 solution was added in quantities of 5.0 to 25.00 cc. All the cultures contained thousands of larvae with the exception of one treated with 20.0 cc. which contained 40, and those with 25.0 cc., which contained 500, 75, and 40 larvae.

One culture to which was added 25.0 cc. of a 1 : 300 solution contained only one larva, but the other cultures treated with a 1 : 300 solution and all treated with a 1 : 400 solution contained thousands.

These results indicate that potassium xanthogenate is most effective when applied as a weak solution; this seems to suggest that it may act at least partially as a contact poison, or, if its lethal property is in the gas that it liberates, that the penetrating power of the gas is extremely low.

Carbon Disulphide

Carbon disulphide was tested in quantities ranging from 0.025 to 25.0 cc. Fig. 2 illustrates how irregular were the results obtained. It is improbable that this irregularity could be ascribed to loss of the chemical from the containers as a gas, especially in those cultures that received the largest quantities as there was no odour of the gas in the constant temperature room. It can more probably be attributed to eggs or larvae or both in small lumps of faeces escaping its action.

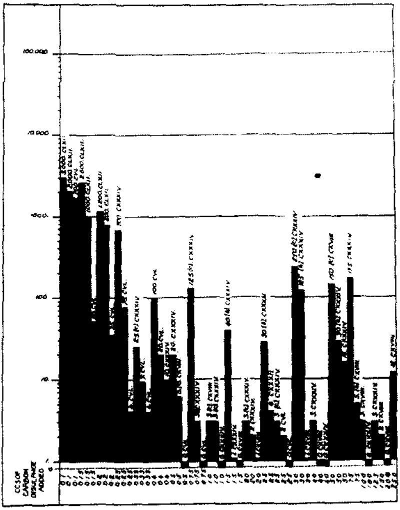

Fig. 2.

Even small quantities of carbon disulphide considerably reduced the number of larvae in most cases. The three cultures treated with 0.33 cc. were practically sterilized, as were a few with lesser amounts, but from two of the cultures receiving 3.0 cc., 250 and 175 larvae were obtained, including some dead, and from cultures treated with 5.0 and 7.5 cc., 150 and 175 larvae

were obtained. These results suggest that approximately 1.0 cc. will cause sterilization under conditions comparable with the technique used. If carbon disulphide gas is the lethal form against Sclerostome larvae, it is probable that, in containers filled by faeces, and if the gas would penetrate the faeces satisfactorily, it would be relatively more effective. However, the irregular results both with potassium xanthogenate and with carbon disulphide suggest that the characteristic of penetrating faeces, which would be so essential in practice, is lacking. The addition of 1.0 cc. of carbon disulphide is equivalent to adding approximately 3.1% of the chemical, by weight.

Zinc Sulphate

The results obtained with zinc sulphate are illustrated in Fig. 3.

This chemical, like other sulphur or chlorine salts of copper, iron, zinc, etc., frequently causes the death of the larvae after they have reached the third stage, although added to the faeces before eggs could have made much development. Whether these larvae, even soon after reaching the third stage, would have been capable of infecting a host is not known.

Zinc sulphate was mixed dry in cultures in quantities of 0.25 to 8.0 gm. When 0.5 gm., or less, was added, numerous larvae, although fewer than in the controls, reached the third stage and continued to live. When 0.75 to 1.5 gm. was mixed in the culture, many larvae reached the third stage, but a considerable proportion died comparatively rapidly. In one out of three cultures treated with 2.0 gm., numerous larvae reached the third stage but died subsequently; similar, but less marked, results were obtained when 3.0, 4.0, and 5.0 gm. were added. An average of these results suggests that about 2.5 gm., equivalent to 6.25%, is the amount of dry zinc sulphate necessary to cause sterilization.

Applied as a 1 : 2 aqueous solution 0.5 cc., or less, caused a comparatively slight reduction in the number of larvae. When from 0.75 to 4.0 cc. was added, numerous larvae were still isolated, but in most cultures many of these rapidly died. The addition of 5.0 cc. limited the number of larvae and also impaired their vitality. From one of three cultures treated with 7.5 cc. many larvae were isolated, but most of these soon died. The addition of 10.0 cc., or more, was effective in causing sterilization. In 7.5 cc. there is approximately 3.0 gm. of this chemical or 7.5% of the weight of the treated cultures.

When 5.0 cc., or less, of a 1 : 4 solution was added, numerous larvae were recovered from all the cultures; when 2.5 cc., or less, was added, the vitality of the larvae did not appear to be affected, but the addition of 3.0 cc., or more, had a marked effect on their viability. One hundred or more larvae were recovered from one of three cultures treated with 7.5, 15.0, and 20.0 cc., but many were dead. In 10.0 cc. of this solution there is about 2.25 gm. of zinc sulphate, or approximately 5.6% by weight.

Less than 5.0 cc. of a 1 : 8 solution did not have much effect on the vitality of the numerous larvae recovered; the addition of 7.5 cc., and more, reduced

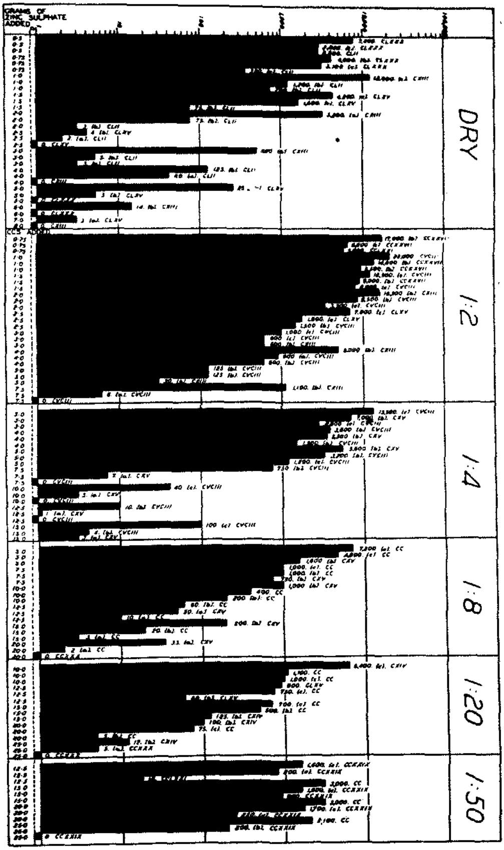

FIG. 3.

their numbers and viability. An average of the results obtained suggests that about 15.0 cc. of a 1 : 8 solution is necessary to cause sterilization. In this quantity there is about 1.8 gm., or 4.5% by weight.

As a 1 : 20 aqueous solution, 10.0 cc. was the minimum quantity that had a consistently marked affect on the numbers and vitality of larvae. The addition of 12.5, 15.0, and 20.0 cc. progressively reduced the numbers, but 25.0 cc., containing slightly less than 1.25 gm., equivalent to 3.1% by weight, is probably necessary to cause complete sterilization.

The results obtained with a 1 : 50 solution, which was tested in quantities ranging from 4.0 to 25.0 cc., were very irregular, both as to numbers and the viability of the larvae. One culture was completely, and another almost, sterilized by 25.0 cc., but from the third, 2100 active larvae were isolated. Smaller quantities of this solution, even 10.0 cc. or less, reduced the numbers of the larvae.

The fact that zinc sulphate is less effective dry, or as a strong solution, seems to suggest that to act on the larvae it must be in very intimate contact with them. Since many survive for some days or weeks after reaching the third stage and then die, it may also be suggested that the poison can penetrate

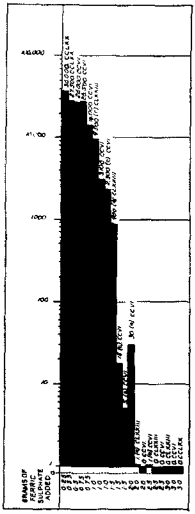

FIG. 4.

the sheath only slowly, and as third stage larvae do not feed in the free stage, it also suggests that it may not be necessary for the larvae to ingest the chemical to be killed by it.

Ferric Sulphate

Ferric sulphate was tested dry only; the results are illustrated in Fig. 4.

Many thousands of active larvae were recovered from the cultures receiving with 0.75 gm. or less. The addition of 1.0 gm. caused a slight reduction, whereas 1.5 gm. caused a considerable reduction in both numbers and viability of the larvae. When 2.0 gm., or 5%, was added no live larvae were recovered. One dead larva was recovered from a culture treated with 2.5 gm., but all the other cultures, treated with up to 8.0 gm., were free of larvae.

Ferrous Sulphate

Fig. 5 illustrates the results obtained with ferrous sulphate.

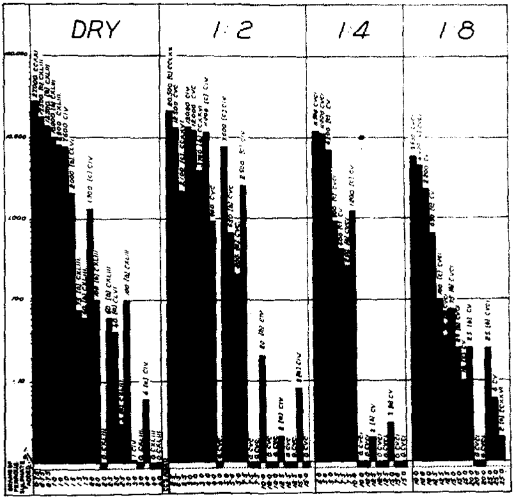

FIG. 5.

This chemical was applied dry in quantities ranging from 0.25 to 8.0 gm. Several thousand larvae were recovered from all cultures treated with 1.0 gm., or less, but some of the larvae obtained from one culture with 0.33 and with 1.0 gm., and from two with 0.75 gm., were dead. The addition of

larger quantities markedly affected the number and viability of larvae. Only from one of three cultures receiving 1.5 and 2.0 gm. were more than a thousand larvae obtained. All other cultures treated with 1.5 gm., and more, yielded only 100, or fewer, larvae—most, or all of which, were dead. An average of these results suggests that at most 3.0 gm., equivalent to 7.5%, is the quantity necessary to effect sterilization.

From one of three cultures treated with 7.5, 5.0, and 4.0 cc., and from all cultures with 3.0 cc., or less, of a 1 : 2 solution, thousands of larvae were isolated; however, some of those from some cultures receiving 2.5 cc., or more, were dead. All larvae from one culture treated with 5.0 cc., were dead, one with 4.0 cc. was free of larvae, as were two with 7.5 cc. The addition of 10.0 cc., and more, sterilized the cultures. This quantity of fluid contains approximately 4.0 gm., or 10% by weight, of the treated faeces.

A 1 : 4 solution was tested in quantities ranging from 2.0 to 25.0 cc. All cultures containing 5.0 cc., or less, yielded thousands of active larvae, with the exception of one treated with 5.0 cc., which yielded a few dead larvae. Three cultures treated with 7.5 cc. and one of the cultures with 10.0 cc. yielded many larvae, but some in each were dead. The others receiving 10.0 cc., or more, were sterilized. In 12.5 cc. of this solution there is almost 3.0 gm. of iron sulphate, or 7.5% by weight.

Applied as a 1 : 8 solution, thousands of larvae were recovered from all cultures to which 10.0 cc., or less, was added, and the vitality of most larvae seemed unaffected. When larger quantities were mixed in the faeces, both numbers and viability were markedly reduced; fewer than 100 larvae were recovered from one of the cultures treated with 12.5 cc. and from all receiving larger quantities. Two cultures were completely sterilized by 20.0 cc. and only a few larvae recovered from the third, as well as from the three treated with 25.0 cc.; most of these larvae were dead. In 25.0 cc. of this solution there is about 3.0 gm.

The results obtained by adding a 1 : 20 solution are not illustrated. It was tested in quantities of 5.0 to 25.0 cc. From all cultures, with the exception of one treated with 20.0 cc. and one with 25.0 cc., thousands of larvae were isolated; even from the two exceptions, 450 and 900 larvae were obtained. In the other cultures the vitality of only a few larvae appeared to have been lowered.

Cupric Sulphate

The results with cupric sulphate are illustrated in Fig. 6.

Very considerable differences exist in the quantities of this chemical necessary to free cultures of larvae and to cause many of the larvae to die after reaching the third stage. This makes an exact interpretation of the results more than usually difficult.

Dry cupric sulphate was added to cultures in quantities ranging from 0.25 to 10.0 gm. The vitality of the larvae in two of three cultures treated with 0.75 and with 1.0 gm., was affected, more especially with the larger quantity.

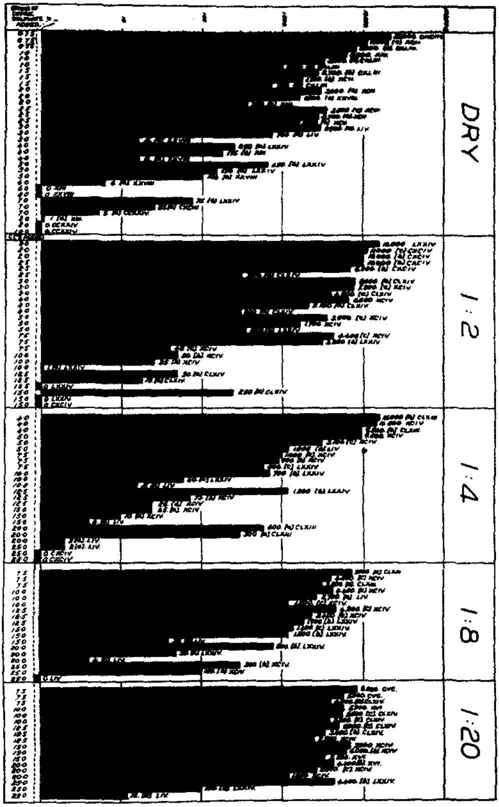

FIG. 6.

When 1.5 gm., or more, was added, some, or all, of the larvae from all the cultures were dead; yet some larvae were obtained from all cultures receiving 7.0 gm. or less, with the exception of two with 6.0 gm. All larvae were dead in one of three cultures treated with 1.0, with 1.5, and with 2.0 gm. and in two of three cultures with 4.0, with 5.0, and with 7.0 gm.; all the larvae

were dead, or the cultures free of larvae, when 3.0, 6.0, 8.0, or 10.0 gm. was added. On an average it is probable that about 4.0 gm., or 10% will kill the larvae, when added to fresh faeces.

A 1 : 2 aqueous solution was applied in quantities of 1.0 to 25.0 cc. A few of the numerous larvae recovered from one of the cultures treated with 1.5 cc. were dead. With the exception of one of three cultures receiving 2.0, 4.0, and 5.0 cc., all treated with 2.0 cc., and more, which yielded larvae, included dead larvae among them. In two of three cultures receiving 3.0 and 4.0 cc., the larvae were all dead; yet, in all cultures to which were added 5.0 and 7.5 cc., and in two of three with 10.0 cc., some were alive. No live larvae were found in any of the cultures treated with 12.5 cc. or more, whereas those treated with 20.0 and with 25.0 cc. were free of larvae. In 12.5 cc. of a 1 : 3 aqueous solution of cupric sulphate there is approximately 5.2 gm., or 13% of the weight of treated faeces.

When cupric sulphate was added as a 1 : 4 aqueous solution, 3.0 cc., or less, did not reduce the numbers or vitality of the larvae very considerably. From the cultures treated with 4.0 cc. many thousand larvae were recovered; however, those from two of the cultures were dead. The addition of 7.5 cc. considerably reduced both the number and viability of the larvae. The larvae from two of three cultures treated with 10.0 cc. were all dead, but a few were alive in all three receiving 12.5 cc. No larvae survived in any treated with 15.0 cc., or more; a few hundred dead larvae were recovered from two of three cultures treated with 20.0 cc. In 15.0 cc. of a 1 : 4 solution of copper sulphate there is slightly less than 3.5 gm., equivalent to approximately 8.7% by weight.

Added as a 1 : 8 solution, 4.0 cc., or less, did not affect the viability of the thousands of larvae recovered, and only a few were affected in two of the cultures treated with 5.0 cc. With a single exception over a thousand larvae were recovered from all cultures treated with 15.0 cc., or less; however, all the larvae were dead in one of three cultures receiving 10.0 and 15.0 cc., and in two of three with 7.5 and with 20.0 cc. In the others to which were added 7.5 cc., or more, there was a considerable loss of viability. An average of these results suggests that approximately 25.0 cc. of a 1 : 8 solution would be necessary to cause complete sterilization. In this quantity of solution there is 3.0 gm., equivalent to 7.5% of the weight of treated faeces.

When a 1 : 20 solution was applied to the faeces all cultures yielded thousands of larvae, with the exception of two of three cultures treated with 25.0 cc. Some of the larvae in about half of the cultures treated with 7.5 cc., or more, lacked viability. One of the cultures to which 25.0 cc. was added was sterilized; the larvae in one of the others were much reduced in numbers and viability, and many of the numerous larvae in the third also died. In 25.0 cc. of a 1 : 20 solution, there is slightly under 1.25 gm.

A 1 : 50 solution was also tested; thousands of larvae were recovered from all the cultures, but with the larger quantities a few of the larvae after reaching the third stage died. The results are not illustrated.

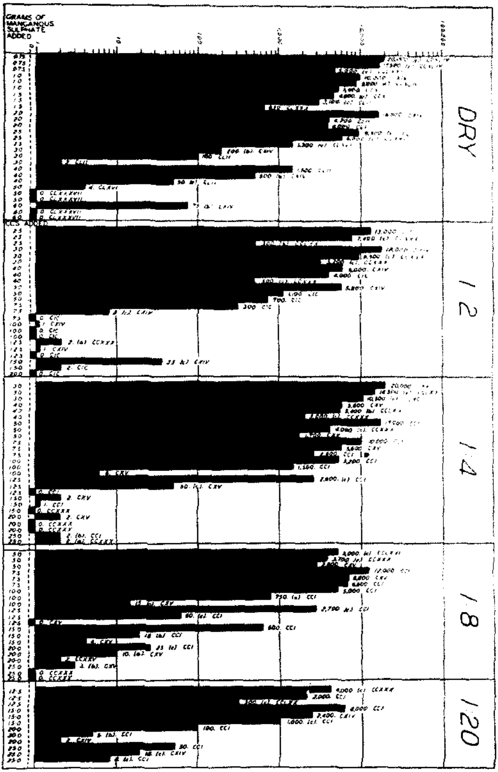

FIG. 7.

Manganous Sulphate

The effects of manganous sulphate are illustrated in Fig. 7.

Dry manganous sulphate was added to the cultures in quantities ranging from 0.25 to 10.0 gm. With the exception of one culture treated with 1.5 gm., which yielded 650 larvae, all those to which 2.5 gm. or less, was added yielded thousands of larvae; one culture to which 4.0 gm. was added also

yielded 1500 active larvae. A few of the larvae from all cultures treated with 0.75 and with 2.5 gm. were dead, as were a few from two of the cultures treated with 1.5 gm. and from one treated with 1.0 gm. The addition of 3.0 gm. markedly reduced the numbers and viability of the larvae, but the addition of 4.0 gm. was slightly less effective. Practically no larvae were recovered from the cultures to which 5.0 gm., equivalent to 12.5%, or more, was added, with the exception of one treated with 6.0 and one with 8.0 gm., from which 75 and 50 larvae were obtained, most of which were dead.

The minimum quantity of a 1 : 2 solution that was tested was 1.0 cc. Thousands of larvae were recovered from all the cultures treated with 5.0 cc., or less, with the exception of one with 2.5, 4.0, and 5.0 cc. However, some of the larvae in two cultures receiving 2.0 and 3.0 cc. and in one with 2.0 and 4.0 cc. were dead. One culture treated with 7.5 cc. yielded 300 active larvae, but the others with 7.5 cc., or more, were practically sterilized, with the exception of one treated with 15.0 cc. from which 35 larvae were obtained, of which a few were dead. These results suggest that, applied as a 1 : 2 solution, nearly 10.0 cc. containing over 4.0 gm., equivalent to about 10% is the necessary quantity to sterilize fresh faeces.

When added as a 1 : 4 solution, with the exception of one treated with 10.0 cc., which yielded only six larvae, all cultures treated with 10.0 cc., or less, and one treated with 12.5 cc., contained thousands of larvae; however, a few dead were included among those from one of the cultures treated with 2.5 and 5.0 cc., and from two treated with 3.0 and 4.0 cc. All cultures to which 15.0 cc., or more, and one to which 12.5 cc. was added, were practically sterilized. In 15.0 cc. of this solution there is nearly 3.5 gm. of manganous sulphate, or 8.75% of the weight of the treated faeces.

Applied as a 1 : 8 aqueous solution of manganous sulphate, 10.0 cc. was the minimum quantity that markedly reduced both the numbers and vitality of the larvae; in two of three cultures treated with this quantity some of the larvae were dead and their numbers were reduced to 750 and 15. The addition of 5.0 cc. also resulted in the death of some of the third stage larvae in two of the three cultures. One treated with 12.5 cc. was free of larvae, but, from the other two, 2700 and 60 larvae, including a few dead, were obtained. The addition of 15.0 cc. reduced the numbers in two of the cultures to 600 and 18, of which many were dead, and, in the third, to four. The cultures receiving 20.0 cc. were practically sterilized, and those with 25.0 cc. were completely sterilized; in the latter quantity there is about 3.0 gm., equivalent to 7.5%.

The addition of a 1 : 20 solution produced somewhat irregular results. From two of three cultures treated with 15.0 and with 12.5 cc. and from all treated with 10.0 cc., or less, thousands of larvae were obtained; however, from two treated with 12.5 cc. and from one treated with 10.0 and 15.0 cc., a few of the third stage larvae were dead. The addition of 20.0 cc. reduced the numbers to 100, 5 and 2; 25.0 cc. further lowered the numbers to 50, 18, and 8, including a few dead larvae.

Sodium Sulphide

Fig. 8 shows the results obtained with sodium sulphide. This chemical was tested dry in quantities ranging from 1.0 to 10.0 gm. The larvae in one of two cultures treated with 2.5 gm. were reduced in numbers and vitality, and 3.0 gm. had a more marked effect on the larvae of one of three cultures. The addition of 4.0 gm. reduced the larvae to 100, 50, and 14, and in the two last cultures, many were dead. When 5.0 gm., and more, was added, equivalent to 12.5% of the faeces by weight, the cultures were practically sterilized.

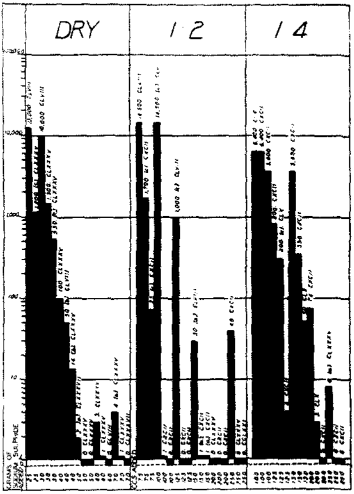

FIG. 8.

The larvae in one culture treated with 5.0 cc. of a 1 : 2 aqueous solution were reduced to 1200. In two of three treated with 7.5 cc. they were reduced to 1700 and 75, a few being dead in both cultures. In one of three cultures treated with 10.0 cc. the larvae were reduced to one, and another culture was free of larvae. From the other cultures treated with 10.0 cc., or less, many thousands of larvae were recovered. One culture receiving 12.5 cc. yielded nearly a thousand larvae, of which a few were dead, one receiving 25.0 cc. yielded 40 active larvae, but the other cultures treated with these, and the intermediate quantities, were sterilized. An average of these results

suggests that about 12.5 cc., which is also equivalent to about 5.0 gm., or 12.5%, is the lethal proportion.

With the exception of one culture to which 3.0 cc. was added, all the cultures treated with 10.0 cc., or less, of a 1 : 4 solution yielded many thousands of larvae. The comparative lack of larvae in the exception, from which only just over 800 were isolated, can probably be ascribed to the presence of the parasitic fungus, *Harpossporium anguillulae*, Lohde; in another culture, *Arthrobotryis obigospora*, Fres. was found, but it had not reduced the larvae to nearly the same extent, 15,000 being obtained.* The addition of 12.5 and 15.0 cc. of this solution considerably reduced the number of larvae. Two of three cultures treated with 20.0 cc. and all three treated with 25.0 cc. were sterilized. In the latter quantity there are approximately 5.5 gm., or 13.75% of the weight of faeces.

The results obtained with sodium sulphide as a 1 : 8 and as a 1 : 20 aqueous solution are not illustrated. With the exception of one culture treated with 20.0 cc. and of two with 25.0 cc. of the 1 : 8 solutions, and of one treated

*The writer is indebted to Dr. H. J. Brodie for the identification of these fungi.

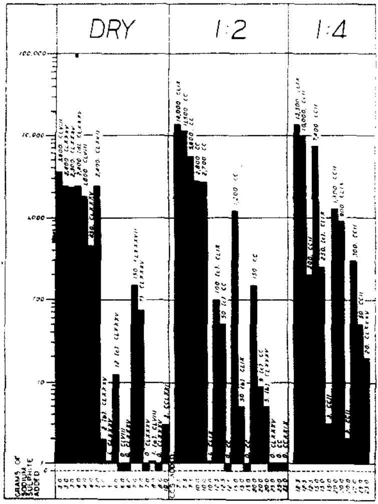

FIG. 9.

with 25.0 cc. of the 1 : 20 solution, all cultures yielded thousands of active larvae; from the exceptions, hundreds of active larvae were obtained.

Sodium Sulphite

The results obtained with sodium sulphite are illustrated in Fig. 9.

Thousands of active larvae were isolated from one of the three cultures treated with 5.0 gm., from two with 4.0 gm., and from all with 3.0 gm., or less. Two cultures receiving 7.0 gm. yielded 150 and 75 larvae, but the third culture was sterilized. Two treated with 5.0 gm., and all with 6.0, 8.0, and 10.0 gm., were practically sterilized. An average of these results suggests that 6.0 gm., or 15% is the lethal proportion.

One culture treated with 15.0 cc., two with 10.0 cc., and all with 7.5 cc., or less, of a 1 : 2 aqueous solution, yielded thousands of active larvae. One culture to which was added 20.0 cc. yielded 150 larvae; one receiving 12.5 cc. yielded 100 and another 50, which included dead larvae. The other cultures treated with these quantities were practically sterilized, one each by 10.0 and by 12.5 cc., two each by 15.0 and by 20.0 cc., and three by 25.0 cc. An average of the results indicated that almost 20.0 cc., containing 7.5 gm., equivalent to over 18%, is the approximate proportion to effect sterilization.

As a 1 : 4 solution, 12.5 cc. reduced the larvae in one culture to 200; 15.0 cc. reduced them in one culture to 250, including a few dead, and in another to three. The other cultures treated with these amounts, or with less, contained thousands of larvae. From the cultures treated with 20.0 cc., 1300, 900, and 2 larvae were obtained; 25.0 cc., which contains slightly under 6.0 gm. of sodium sulphite, reduced the larvae to 300, 50, and 20.

All cultures treated with a 1 : 8 and with a 1 : 20 solution, yielded many thousands of active larvae, with the exception of one of three cultures treated with 25.0 cc. of the 1 : 8 solution, which yielded only 20 larvae, and one of three treated with 25.0 cc. of the 1 : 20 solution, which yielded 1400 larvae, of which some were dead. The results with these solutions are not illustrated.

Sodium Sulphate

Fig. 10 illustrates the results obtained with sodium sulphate.

It was tested dry in quantities ranging from 1.0 to 14.0 gm. All cultures made with 4 gm., or less, contained thousands of active Sclerostome larvae. The addition of 6.0 and 7.0 gm. reduced the numbers of larvae and also the viability of a few of them. The numbers were further reduced in two cultures treated with 8.0 gm. and the third was free of larvae. One culture was completely and another almost sterilized by 10.0 gm., and most of the larvae obtained from the third were dead; this quantity is equivalent to 25% of the weight of faeces.

When a 1 : 2 solution was applied, one culture was sterilized by 15.0 cc. and all by 20.0 and by 25.0 cc.; those in which 15.0 cc. was used, and less, contained thousands of larvae. In 20.0 cc. of this solution of sodium sulphate, there is the equivalent of approximately 19% of the weight of faeces.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested; the results are not illustrated. The addition of 25.0 cc. of a 1 : 4 solution reduced one culture to 900 larvae, but all the other cultures made with this solution contained thousands of larvae.

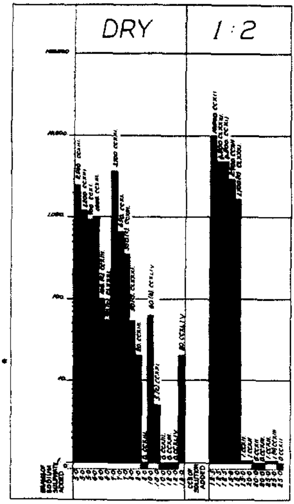

FIG. 10.

Magnesium Sulphate

The results obtained with magnesium sulphate are illustrated in Fig. 11.

Applied dry, all cultures treated with 6.0 gm. or less, contained thousands of active larvae; those treated with 7.0 and with 8.0 gm. also yielded many, although fewer active larvae. The addition of 10.0 and 12.0 gm. further reduced the numbers of larvae, while both quantities sterilized one culture. A few larvae were recovered from most of the cultures treated with 14.0 gm. (equivalent to 35% of the weight of the faeces), 16.0, and 20.0 gm., but all were dead, except those from one culture treated with 16.0 gm., which yielded 125 larvae.

All the cultures treated with 15.0 cc., or less, of a 1 : 2 solution yielded many thousands of larvae. Two cultures were sterilized by 20.0 cc. and two by 25.0 cc., but the third treated with 20.0 cc. yielded 350 larvae, some of

which were dead, and the third treated with 25.0 cc. (containing almost 10.0 gm. of magnesium sulphate, equivalent to 25%) yielded 2100 larvae.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested. Fewer larvae were found, of course, in the cultures treated with the largest quantities of fluid, but all the cultures contained thousands of active larvae. These results are not illustrated.

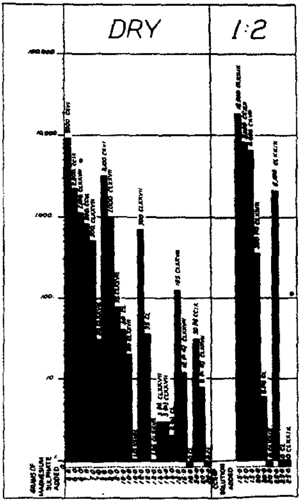

FIG. 11.

Zinc Sulphide

The results obtained with zinc sulphide which was tested dry only, are not illustrated.

All the cultures to which 10.0 gm. or less was added yielded many thousands of active larvae. The cultures treated with 12.0 and 14.0 gm. also yielded many thousands, most of them active, but in four of the five cultures, a few were dead. From one culture treated with 16.0 gm. only 100 larvae were obtained, many dead, but from the other two cultures treated with this amount, 41,000 and 2200 larvae were isolated, of which only a few were dead.

The addition of 20.0 gm. (50%) was more effective; one culture yielded only 30 dead larvae, another 200, of which a considerable proportion were dead, and the third 2200, including a few dead.

Ferrous Sulphide

Ferrous sulphide was tested dry only; it, like ferrous oxide, which was discussed in a previous paper (34), appears to have no lethal value. Ferrous sulphide was tested in quantities ranging from 1.0 to 20.0 gm. and compared with the controls there appeared to be no significant decrease or increase in the number of larvae isolated, or in their vitality.

Sulphur

Flowers of sulphur was tested in quantities ranging from 1.0 to 20.0 gm.; quantities of 10.0 gm., or less, were tested in duplicate, those over 10 gm., in triplicate.

TABLE I

CONTROLS FOR CULTURES TABULATED IN FIGS. 1–11

Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated
	1935				1936—*Conc.*		
XIII	7 May	31	11,000	CLXXIII	26 November	60	37,000
XVI	22	22	25,000	CLXXVI	5 December	52	71,000
XXIII	*9 July*	*16*	*93,000*	*CLXXVII*	*5*	*58*	*33,000*
XXIV	9	18	48,000	CLXXIX	8	58	98,000
XXVI	9	18	70,500	CLXXX	9	57	69,000
XXVIII	11	19	2,700	CLXXXI	9	57	72,000
LIV	10 December	17	6,200	CLXXXV	23	54	16,000
	1936			CLXXXVII	30	48	11,000
LXXIV	24 January	24	11,500		1937		
XCII	11 March	22	26,000	CXCII	8 January	55	12,000
XCIII	19	14	27,000	CXCIII	11	45	35,000
XCIV	19	21	35,000	CXCIV	12	51	31,000
CIV	7 April	23	21,500	CVC	12	55	22,500
CV	7	24	19,500	CVCI	19	48	29,500
CXIII	1 May	22	70,000	CVCII	20	47	31,000
CXIV	1	22	33,000	CVCIII	22	52	37,000
CXV	1	37	13,000	CIC	26	48	50,000
CXVI	7	15	36,000	CC	27	50	26,500
CXVII	7	23	26,000	CCI	28	56	40,000
CXVIII	12	21	2,000	CCII	29	48	19,000
CXXXII	12 June	22	8,500	CCVI	12 February	52	36,000
CXXXIV	17	37	40,000	CCVIII	18	49	55,000
CXXXIX	9 July	18	43,000	CCIX	18	46	40,000
CXLIII	31	74	18,500	CCX	23	44	35,000
CXLIV	31	77	13,500	CCXI	24	50	32,000
CVL	3 August	81	22,500	CCXII	1 March	38	51,000
CL	6	99	4,600	CCXIII	2	48	62,000
CLI	7	98	1,100	CCXXI	16	23	26,000
CLII	7	108	5,000	CCXXIV	17	65	41,000
CLVI	26 October	39	14,500	CCXXVI	30	48	34,000
CLVIII	29	43	39,000	CCXXVII	1 April	51	42,000
CLIX	30	44	71,000	CCXXIX	5	56	33,000
CLX	2 November	42	15,500	CCXXX	6	55	22,500
CLXII	5	49	39,000	CCXLIV	20 May	25	49,000
CLXIII	9	45	55,000	CCVLI	21	34	29,000
CLXIV	11	43	24,500	CCLXX	22 July	74	34,000
CLXV	12	53	43,000	CCLXXI	23	83	16,000
CLXVI	16	45	23,500	CCXC	28 October	28	46,000

Sulphur appears to have no value in reducing the numbers of larvae. In a few cultures made with various quantities, a few of the recovered larvae were dead, but there appeared to be no correlation between the amount applied and the subsequent death of the larvae. In fact, the results suggest that by controlling parasitic fungi or other harmful factors, the number of larvae recovered may be increased. In 20 of the 30 cultures more larvae were recovered from the treated than from the control cultures. The controls were those for series XIII, XXIII, XXVI, CXLIV, CLXV (see Table I). Five cultures contained more than three times as many larvae in the treated as in the control cultures, and ten, more than twice as many; naturally, the largest increases tended to be in the series with the fewest larvae in the controls—13,500 and 11,000. The average number of larvae in the treated cultures was 42,400, an increase of just over 25% of the number that the controls show, would have been in 30 untreated cultures.

Conclusions

Potassium xanthogenate, unless its cost were too high, might be used under some conditions as a lethal agent against the free-living stages of Sclerostomes, since it is fairly effective as a weak solution. Carbon disulphide, unless it proves more effective as an emulsion, does not appear to have sufficient penetrating power. The other salts discussed in this paper all seem too weak to be of value alone. The "delayed action" of some, however, suggests that, if they were brought into intimate contact with the larvae through the action of another chemical, they might have some value. Unfortunately, much more work must be done before any constructive proposals can be made on this basis.

Acknowledgments

It is a pleasure to be able to add the names of Messrs. Rohm and Hass Co. Inc., of Philadelphia, and of Messrs. The Dow Chemical Co., of Midland, Michigan, to the list of firms who have given chemicals for this work, and to be able to record the writer's gratitude to them.

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PARASITES OF SOME CANADIAN SEA MAMMALS[1]

By L. L. Lyster[2]

Abstract

Four nematodes, two acanthocephalids and specimens of the cestode genus *Diphyllobothrium* are reported from seals and white whales in Arctic and temperate coastal waters of Canada. *Phocascaris netsiki* sp. nov. is described from *Phoca hispida* and observations on flagella in *Anisakis simplex* are recorded.

To the greater number of Canada's Arctic and sub-arctic inhabitants the sea-mammals supply staples both of food and clothing. In the course of our survey of parasites a considerable amount of material has been collected post-mortem, from these animals. Seals are represented by collections from several of the eastern Arctic posts. This material (as was the single parasite-free walrus examined) was secured chiefly with the co-operation of the Royal Canadian Mounted Police and the Hudson's Bay Company who sent formalized viscera to the Institute. In addition, collections were made by Dr. Parnell in 1934 (10) and by the author in 1939. White whale parasites, collected in the Gulf of St. Lawrence, were supplied to us by Dr. V. D. Vladykov and viscera of whales have also been received from the eastern Arctic posts.

Formalization of viscera leaves much to be desired from the standpoint of the condition of the material collected, but it has made available resources and collecting points that would not otherwise have been reached. The sources of the present material, as listed with the hosts, have been mapped in an earlier paper (4).

Parasites recovered during the survey included nematodes, cestodes and acanthocephalids. Trematodes are conspicuously absent; our correspondence files include reference to "liver flukes" of seals at Cape Smith, but our post-mortem studies on livers from this area, though showing fatty degeneration and necrosis, have produced no trematodes. Personal notes also include a report by a Cape Smith native who described white rough livers in local seals, a condition which, according to the Esquimaux, renders the entire carcass unfit for food.

In fish-eating seals and white whales, nematodes invariably occurred in the stomach in close association with the partially disintegrated portions of fish

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found there. In many cases these lodged so firmly in the fish muscles that it was necessary to dissect them out. These nematodes are adult forms and therefore not introduced with the food. Whether they feed on the fish ingested by their host, act as symbionts serving to break up the bolus, or whether there is some more obscure relationship, remain interesting speculations. Extracorporal digestion among ascarids has been noted by Hoeppli (7) among *Contracaecum* sp. in seal stomachs. The nematodes he studied were actively parasitic on the host, but the fact is established that they are capable of producing histolytic changes in an external medium. The nematodes concerned must be highly anti-proteolytic; apparently healthy individuals are to be found in the oesophagus, stomach, and small intestine of the more heavily infected animals.

Nematodes

Three species of different genera are included in our seal collections and one species is reported from white whales.

Porrocaecum Railliet & Henry, 1912.

The most common parasite of the bearded seal belongs to the genus *Porrocaecum* on the criteria of the presence of a single intestinal caecum and dentigerate lips. The single species of the genus reported from Arctic seals has been discussed by Baylis (1) who reviewed the earlier studies and material and differentiated the species largely on the structure of the lips and alimentary tract. Since establishment of the genus, many major points of species differentiation have become generic and it is difficult to refer the present material to the species *P. decipiens* described by Baylis; a detailed description is accordingly given.

The male is 9 to 11 mm. long by 0.5 to 0.6 mm. wide in greatest measurement. The female is about 15 mm. long by 1 mm. wide, with a maximum specimen of 37 to 1.25 mm.

The two subventral lips are triangular in shape, not unlike a conventional heart, apex toward the base, anterior margin cut medially to make two prominent lobes. The pulp of the lip follows the same outline, leaving a semitransparent margin. A prominent single papilla is situated at the apex of the triangle. The anterior margin of the dorsal lip is similarly cleft, but the base of the lip is wider than the free portion. About midway the length of the dorsal lip there is an external thickening behind which two papillae are situated. No interlabia are present. Dentigerous ridges vary in arrangement and extent. In some specimens they are to be seen only at the contact of the anterior lobes; in others they are continuous throughout the anterior margins while in one case they outline the lateral margins of each lip. In some specimens it was impossible to find any evidence of dentigerous ridges, though other features were constant. These structures were never very conspicuous.

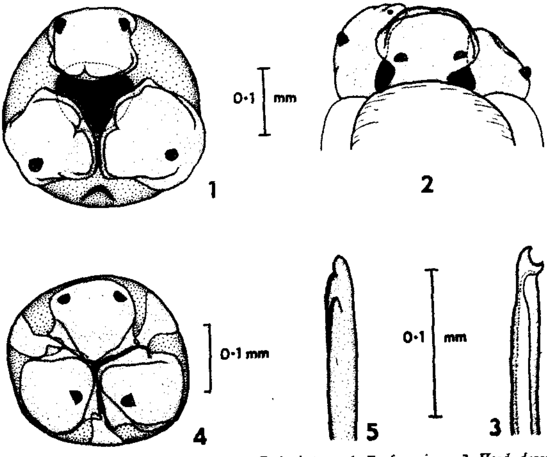
The oesophagus is divided into the usual two structurally different parts, continuous with each other, and opening without change of direction into the

intestine. The anterior portion is 1.7 mm., the posterior 0.15 mm., in length. At the point of origin of the intestine rises a diverticulum which runs anteriorly to a point approximately in line with the limit of the glandular oesophagus, a distance equal to about one-fortieth the body length.

The female tail is bluntly rounded with a small terminal knob. The vulva is one-third, or somewhat less, the body-length from the anterior end. The genital structures are as figured by Baylis.

The male body terminates in a sharp point but is given a blunt appearance by a cuticular wing, 0.03 mm. at its widest point, which is continuous around the end of the body, from a point roughly in line with the cloacal opening. The tail is 2.5 mm. long carrying seven papillae; three are lateral and paired, one is medial and almost terminal. More or less parallel with the spicules four pairs of papillae are situated on either side of the cloaca and a single large one is central and just anterior to this opening. The greatest number of pre-anal papillae found in any of our specimens was 52 pairs. For some distance anterior to the cloaca they occur in a regular line parallel to the sides of the body, but about the thirtieth they tend to become scattered into a general plan of two rows in which the papillae alternate in pairs.

The spicules are equal, 1.5 mm. long. They are straight to the tip, with lateral alae which curve ventrally, meeting just before the end. Terminally



FIGS. 1 – 3. *Porrocaecum decipiens ex E. barbatus. 1. En face view. 2. Head, dorsal view. 3. Spicule.* FIGS. 4 – 5. *Contracaecum osculatum ex P. vitulina. 4. Head, en face view. 5. Left spicule.*

the spicule broadens and a semilunar indentation gives it a clawed appearance.

These specimens are notably smaller than those discussed by Baylis. This is a fact for which it is difficult to find an explanation; even although no well-developed eggs were noted, it seems unlikely that the increase in size to complete maturity, would be as much as two to three times. Relative measurements, a more satisfactory criterion, correspond with those given by him.

The main lips are not identical with those discussed by Baylis and there is variation in the numbers of pre-anal papillae; these two factors are considerably influenced by fixation within the limits of the present differences.

Because of general head structure, papillary arrangement and, notably, on biological grounds, the present material has been considered to be the species *P. decipiens*. The variations noted are not of sufficient significance to allow specific differentiation and Arctic seals are considered as carrying the single species.

Less mature specimens of the same species were recovered from the stomach of most of the individual animals studied. These were usually attached to the mucosa in bundles of about 20 worms. The largest specimen occurring in this site measured 1.8 by 0.08 mm.; the oesophagus was 0.28 mm. and the caecum 0.04 mm. in length. The larger number of these forms was about half this size. Specimens found unattached in the stomach included many enclosed in their larval sheath, and adults were also recovered here. A single specimen was taken from the oesophagus of a Jar Seal *(Phoca vitulina)*.

Most specimens of *Erignathus barbatus* that we have examined are parasitized by this nematode; these have come from ports on either side of Hudson Strait. In addition, we have records of it from *Phoca groenlandica*, Stupart Bay, from "Seals", Havre St. Pierre, Que., and Craig Harbour, and from a "Jar Seal" taken at Wolstenholme. Because of feeding habits, etc. of *P. vitulina* the "Jar Seal" would seem to be a mistaken identification of the host.

Contracaecum Railliet & Henry, 1912.

This genus has been fully discussed by Baylis (3), who lists three species from pinniped hosts. The present material corresponds most closely to his description of the northern species *C. osculatum*. The dorsal lip is of relatively uniform width with sharp cuticular lobes, the oesophagus is cylindrical, the spicules over nine mm. long; these are features on which he bases the identification. In over-all measurements and in most details of structure the present specimens correspond exactly with that species. Some differences in detail, however, must be noted.

In the male specimens, averaging 40 mm. long by 1.8 mm. wide, in no instance were there as many as 50 pre-anal papillae; they numbered about 40. Wrinkling and shrinking of the cuticle obscures these structures and will account for at least some of the difference. The spicules were slightly longer than Baylis records, ranging up to 10.3 mm. The spicular alae do not reach the end of the spicule and that on the right does not extend so far as that on the left.

In the female, up to 60 mm. long by 2.5 mm. wide, the vulva is situated at about the limit of the anterior sixth of the body. The oviduct divides, each portion entering a branch of the uterus, at about one-third the body length. Near this point the ovaries rise and proceed backwards to within 9 mm. of the posterior end (in a 60 mm. specimen), then run forward about 5 mm. to meet the uterine tubes.

In spite of the variations (which are readily attributable to different methods of fixation) since over-all characteristics and particularly the structure of the lips, are so closely identifiable with Baylis's detail for *C. osculatum*, it is to this species that the present material has been assigned.

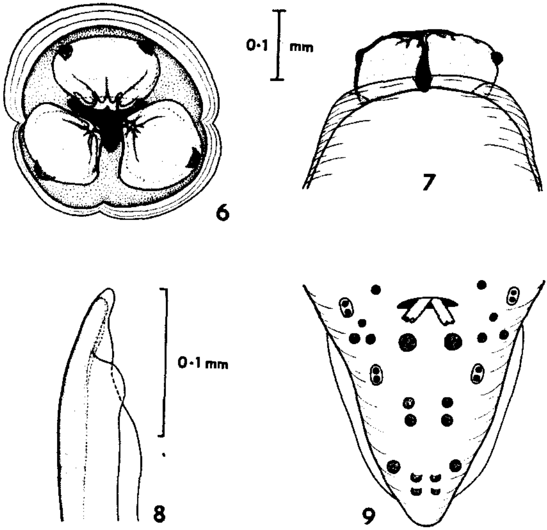
The species has been recorded in the survey from *P. hispida* and *P. vitulina*. It is found from Dundas Harbour, Devon Island, and Craig Harbour in the north to Havre St. Pierre, Que., in the south. Intermediate points of collection have been Lake Harbour, Clyde River in Baffin Island, and Stupart Bay and Wolstenholme on Hudson Strait.

Phocascaris Höst, 1932

This genus has hitherto included the single species *P. phocae* reported by Höst from *P. groenlandica* (8). It is very like *Contracaecum* in conformation of alimentary tract but because of modified and dentigerate lips it cannot be plaçed in that genus. *Phocascaris*, accordingly occupies a valid place among the Anasikinae.

Several specimens collected from seals in the Canadian north show the features that characterize this genus. None of them, however, conform exactly to Höst's description of the species (8). In *P. phocae* the anterior margins of the lips are formed by four labial folds decorated with dentigerous ridges and each subventral lip carries a large and a small papilla. In the present material the anterior margins of the lips are entire with an occasional exception in the case of the dorsal lip when a medial indentation may occur. Dentigerous ridges are continuous from about the midlateral areas of each lip. Somewhat removed from the margin, six labial folds are conspicuous, the outermost pair being closely applied and outlined by dentigerous structures giving the effect of four submarginal scallopings. A single large papilla is to be seen on each subventral lip and a pair of double papilla lateral to the male cloaca are further features that distinguish this material from *P. phocae*, in which no comparable structures were seen. These differences and the size are sufficient to make it impossible to identify these specimens as belonging to Höst's species, and it is accordingly necessary to create a new species to designate our collection. For this the author proposes the name *P. netsiki* sp. nov.

The female is 30–76 mm. long with a maximum width of 0.6 to 1.2 mm.; male specimens measure 20 to 51 mm. long. The oesophagus, about one-tenth to one-fifteenth the body length, often coils upon itself midway in its course. The proventriculus about one-eighth the length of the oesophagus proper, opens without change of direction into the intestine. An intestinal



FIGS. 6 – 9. *Phocascaris netsiki ex P. hispida.* *6. En face view.* *7. Head, ventral view.* *8. Spicule.* *9. End of male, showing peri-cloacal and terminal papillae.*

caecum is equal to or only slightly greater than the proventriculus. A wide oesophageal diverticulum runs posteriorly a distance equal to half the length of the oesophagus proper, terminating in a sharply recurved distal point. Inconspicuous lateral cephalic alae occur.

The female genital complex varies in organization from that described for *P. phocae.* The oviduct opens by a narrow constriction into a Y-shaped uterus. The two posterior branches of the uterus are joined by the much smaller ovaries which enter dorsally and just short of the ends of the branches. The ovarian tubes follow a convoluted course posteriorly, almost to the anus, then run directly forward to the level of the oviduct. The vulva is situated about one-fifth the body length from the anterior end. The eggs are spherical or slightly ovoid, thin-shelled, containing a segmented ovum; they are about 0.08 mm. in diameter.

The male spicules are long, sub-equal and simple. They are alike in structure. A strongly chitinized dorsal rod is continuous ventrally with a semitransparent hollow tube. This latter divides about 1 mm. from the end to form two lateral alae. One of these is extended around the end, the

other is discontinued just anterior to the end of the spicule. In a specimen 51 mm. long the left member was 5.0 mm. and the right 5.5 mm. long. They average about one-tenth the body length. Five pairs of single and one pair of double papillae decorate the tail and a similar number are peri-cloacal, of the last a pair of double papillae are laterally in line with the cloaca. Fifty pairs of single pre-cloacal papillae follow a regular course parallel to the sides of the body. Narrow lateral caudal alae are present.

In both sexes the cuticle is finely striated throughout; these striae coupled with close cuticular folds, form a distinct collar around the base of the lips.

Infections with *P. netsiki* were never heavier than five to ten worms in a host. They were found in *Phoca hispida* from Craig Harbour, Clyde River, and Lake Harbour, N.W.T., and in a "seal" from Cape Smith, N.W.T.

Anisakis Dujardin, 1845

Several thousand ascarids collected from the alimentary tract of *Deliphinapterus leucas* are identifiable as belonging to the genus *Anisakis*, on a basis of absence of alimentary diverticula and interlabia and presence of dentigerous ridges and a two-part oesophagus.

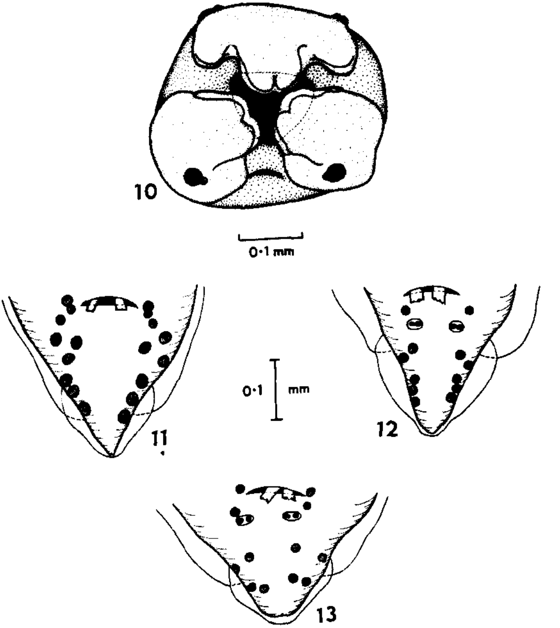
From marine mammals eight species of this genus have been described, but marked confusion of early descriptions and later synonymities make specific determination almost impossible. Three species, *A. similis*, *A. rosmari* and *A. physeteris* have been reported from carnivorous mammals, and one, *A. insignis*, from a freshwater dolphin. The remaining four species have been described from various porpoises and small whales, and it is among these that most confusion has arisen. *A. dussumierii* Van Beneden, 1870, was first described as *A. simplex* by Dujardin; *A. typica* Diesing, 1860, has been listed as a synonym of *A. dussumierii* by Stiles and Hassall (11) and *A. kukenthalii* Cobb, 1888, has been proposed as a synonym of *A. simplex* Rudolphi, 1809, by the same authors.

These four species are not only poorly differentiated, but they have a great deal in common. The problem of assigning the present material to a place in the genus is one of selecting constantly differential characteristics and interpreting them to apply to our specimens. Table I, correlating the measure-

TABLE I

COMPARATIVE MEASUREMENTS OF *Anisakis* FROM MARINE CETACEA (MM.)

Male	Female	Oesophagus I	Oesophagus II	Spicules	No. post. papillae	No. prepapillae	Width striae	Species
31–70	37–90	4	1.25	3.9 and 0.96	6–10	75 plus	32μ	*typica*
79	70–100	5.5	1.5	2.7 and 1.5	8–10	?	30μ	*dussumierii*
37–130	79–200			1.68	6–8	50 plus	23μ	*simplex*
70–90	80–100	5.1	2.5	2.3 and 1.7	7–8	90	30μ	*kukenthalii*
60–90	63–110	5.4	1.6	2.0 and 1.4	7–9	56–90	23μ to 38μ	Present form



FIGS. 10 – 13. *Anisakis simplex ex D. leucas. 10. En face view. 11 – 13. End of male showing progressive modifications in papillary arrangement.*

ments given for cetacean-parasitizing *Anisakis* in the literature (notably, in Stiles and Hassall (11)), illustrates the inadequacy of measurement in specific diagnoses. (A similar table is used by Baylis (2) in establishing his species *A. physeteris*). The minimal measurements of 31–79 mm. in the male and 37–80 mm. in the female and the maximal measurements of 70 to 130 mm. and 90 to 200 mm. in the female will not exclude a single species. Similarly measurements of structures and papillae counts are non-differential. If any species is in any way distinct it is *A. typica*, on a basis of insignificantly smaller size, and its separate identity from *A. dussumierii* has already been questioned. In the same way, arrangements of structures do not offer a means of separation. No detail of labial structure has been held significant except the tendency for greater distinction between the basal and lobate portions of the dorsal lip illustrated by Krabbe (9) and by Stiles and Hassall working with "*A. typica*".

The female worms of the group are undifferentiable from descriptions. The variations in spicule length and papillae arrangement in the males are limited to differences within definable limits into which all species will fall.

Because they are found in a closely related group of circumpolar animals of similar habit, and because there seem to be no satisfactory morphological criteria for recognizing distinct species, it seems unlikely that these four members of the genus are dissimilar beyond the insignificant variations that are shown within most nematode species. This view is further borne out by the considerable differences that have been noted between these four members of the genus on the one hand and *A. similis*, *A. physeteris*, and *A. rosmari* on the other, a differentiation that is to be anticipated on biological and ecological, quite apart from morphological grounds.

For these reasons the present material has been assigned to the species *A. simplex* which is here considered to have as synonyms *A. kukenthalli*, *A. typica*, and *A. dussumierii*.

Males in our collection measure 60 to 90 mm. by 1.7 to 2.4 mm., females 63 to 100 mm. by 2.0 to 2.6 mm. The oesophagus has an anterior muscular portion averaging about 5.5 mm. long, and a posterior, usually laterally sigmoid, proventricular portion 1.5 mm. long, or slightly greater. Cuticular striae 23 to 38μ apart are continuous throughout the body length. The three lips follow the usual typical pattern; a crescentic dorsal lip 0.24 by 0.12 mm. bears a pair of anterior projections medially; two subventral lips are more nearly round in outline, with a pair of lobes reduced in extent but similar to those of the dorsal lip. Dentigerous ridges outline the opposed margins of these lips. The dorsal member carries a pair of papillae at the lateral corners and each subventral lip is decorated with a pair of ventral papillae. A pair of cervical papillae is about 1.0 to 1.5 mm. removed from the base of the lips.

The vulva of the female opens just in front of the middle of the body (45 mm. from anterior end in a specimen 105 mm. long). An oviduct about 12 mm. long loops longitudinally before entering the bicornate, posteriorly directed uterus about 33 mm. long. Each uterine branch is joined by a long, slender and highly convoluted ovary which runs backward for a short distance, then to the forepart of the body well anterior of the vulva. Intrauterine eggs are subspherical, 0.045 to 0.5 mm. in diameter.

The male spicules are slightly curved, slender, dissimilar; the left is 2.0 mm. or slightly more, the right 1.2 to 1.4 mm. in length. Sixty to ninety pairs of pre-anal papillae have been counted, the greater number of specimens having a larger number of these structures.

The tail of the male is relatively short, 0.18 to 0.22 mm. long. A narrow ala, continuous around the end of the tail is about 0.04 mm. in marginal extent. It meets lateral alae 0.02 to 0.03 mm. wide that terminate just anterior to the cloaca. Pre-cloacal papillae number 60 to 90 pairs reaching an anterior limit 5.0 mm. from the end of the body. Post-cloacal papillae

numbering six to eight pairs, differ in arrangement. Nearest the tip in some specimens three pairs occur closely in line parallel to the body margin; a fourth marginal pair is anterior to these and a fifth pair lies anteriorly toward the midline. Two pairs extend from below the lateral margins of the cloaca toward the alae and just at the corners of the cloaca two pairs are in close contact. The third of the three posterior pairs is not infrequently absent; in specimens showing this condition the papillae are arranged in well defined groups of two, of which the immediately post-colacal group sometimes includes a pair of double papillae toward the midline. No other differences may be noted in association with these variations in papillary arrangement.

In these helminths the male genital tract shows a noteworthy condition. The vas deferens, immediately after its origin, is folded in a longitudinal sigmoid curve from which it proceeds directly to the cloaca. Within a limited area at the beginning of the direct portion of the deferens the lining epithelium is decorated with hair-like processes. These occur in clusters of about 30 placed about 25μ apart in longitudinal rows. The rows, 35μ apart, are laterally staggered, so that the groups of processes alternate around the organ. The individual processes are relatively uniform in length, from 60 to 65μ; 25 groups of these structures may be seen in cross section.

The material now available has all been preserved in formalin for a considerable time and it is not possible to determine whether these structures are, as their appearance strongly suggests, flagella. In describing "*Ascaris kukenthalii*" Cobb (5) noticed similar forms. His descriptions and illustrations prove that the structures he saw were identical with those in the present material. Apparently using living material, he reported motility for the processes and even attributed a definite function to them, that of assisting the movement of the spermatozoa on their way down the vas deferens. He says in part: "This" (the epithelium of the posterior part of the vas deferens) . . . "is provided with bundles of strong cilia which remind one of the not dissimilar elevations of the epithelium in *Ascaris lumbricoides*. In that case, however, the elevations are branching and show, according to Schneider and Lueckart, only amoeboid movement. Here, on the other hand, only bundles of often true cilia occur to which I must attribute a very active movement, not only because they show the composition of real cilia, but also, mainly because the free ends are directed posteriorly, and we find all the spermatozoa assembled in a central longitudinal bundle. In the space between the axial mass of sperms and the wall of the duct, i.e., in the space in which the cilia move, there are no spermatozoa."

Then follows a discussion of the grouping and extent of these structures, parallel in every way with those the author has noted. The observations of the author are further comparable in that the spermatozoa are invariably swept to the centre of the duct as Cobb has described.

Hetherington (6) in a discussion of ciliation quotes Cobb in an 1898 paper in which he discusses the motility of structures in the seminal vesicle without apparent reference to the worm in which he noted the condition; it would

seem that Cobb had in mind the observations quoted here and of which Hetherington was apparently unaware. The length of these processes, as well as their structural arrangement in distinct tufts, readily suggests their classification as flagella purely on the basis of appearance and arrangement. If, then, the evidence of Cobb's paper for their motility is accepted it must be concluded that this nematode species, at least, has flagella.

By far the greater bulk of our *D. leucas* material originated in the Gulf of St. Lawrence. Viscera of only three white whales have been available from other sources. Of these, one from Southampton Island, N.W.T., was parasite-free, one from Craig Harbour, Ellesmere Island, furnished a single immature *Anisakis* and the third, with origin at Lake Harbour, Baffin Island, was parasitized by a few immature *Anisakis* sp.

Acanthocephalids

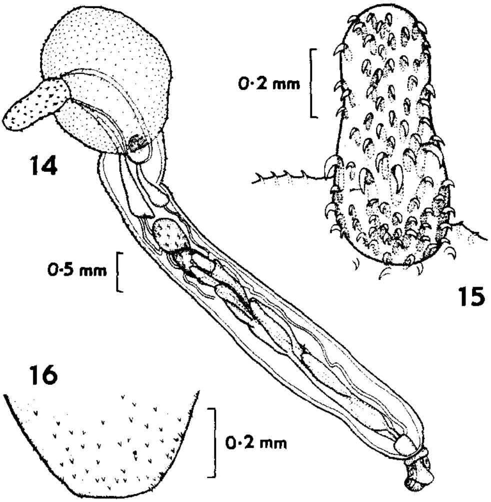
Corynosoma Luhe, 1911

Fixed firmly in the mucosa of the small intestine of seals, acanthocephalids of the species *C. strumosum* were collected from *Erignathus barbatus* taken at Cape Smith, Craig Harbour, Clyde River, and Pond Inlet, and from ***Phoca hispida*** at Cape Smith, Lake Harbour, Payne Bay, Stupart Bay, and Wolstenholme. *C. semerme* was found only once in a specimen of *E. barbatus* taken at Lake Harbour in August, 1939. In an earlier paper (4) an infection of *C. semerme* from dogs at Craig Harbour is recorded; though some of the seal acanthocephalid material from this post is very badly preserved there is little doubt that the specimens are *C. strumosum* rather than *C. semerme*. Though none of the Arctic material from *Delphinapterus leucus* included acanthocephalids, white whales in the Gulf of St. Lawrence were commonly parasitized by *C. strumosum*.

Corynosoma strumosum Rudolphi, 1892

Males are about 7 mm. long by 1.2 mm. wide in the region of the testes; females slightly larger when gravid. The body is straight, with almost parallel sides, up to an expansion at the anterior end where the body width is doubled. The proboscis is slightly barrel-shaped 0.85 mm. long by 0.35 mm. at its widest points. Spines in 18 rows decorate the organ; these are smallest at the base of the proboscis, extending about 0.04 mm. from the cuticle; at the fourth row they increase in size to 0.09 mm. then gradually decrease in size until, at the anterior tip, they are about 0.05 mm. The root of these spines is always about one-fourth greater than the free portion.

The proboscis narrows to the anterior third, then increases in size toward the base. The anterior expanded portion of the worm is thickly armed with small spines set deep in the cuticle; these increase in size at the base of the proboscis, and are continued into the body of the worm to a maximum point just below the posterior testis in the male and a corresponding position in the female. Spination is renewed terminally in the male. The proboscis sheath is relatively short, reaching a point just behind the testes; the lemnisci are truncated, not reaching the testes.



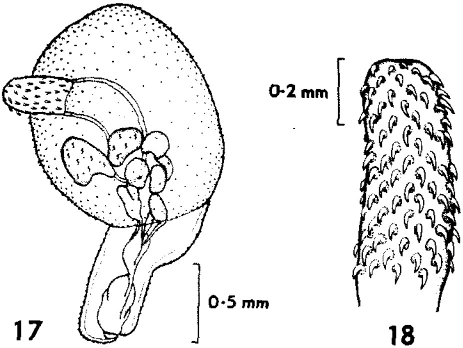
FIGS. 14 – 16. *Corynosoma strumosum ex E. barbatus.* 14. *Entire worm, male.* *15. Proboscis.* *16. Detail of terminal spination, male.*

The two testes of the male are roughly rectangular in shape, 0.5 by 0.3 mm., longitudinally colinear and in close contact with each other. The numerous cement glands form a compact mass running from the level of the middle of the posterior testes to the posterior end. Retinaculae follow an irregular course posteriorly from the brain. A conspicuous copulatory bursa is present. The female, apart from sexual structures, including the absence of terminal spines, is not dissimilar from the male. The body contains many embryos about 1.08 mm. by 0.5 mm. in size.

Corynosoma semerme Forssell, 1904

Males measure about 1.3 to 2.0 mm. by 0.9 to 1.0 mm.; females 2.8 to 3.0 mm. by 2.0 to 2.2 mm. The present specimens have been found to compare with the *C. semerme* material from dogs identified by Dr. Van Cleave. They have been assigned to this species on the criteria of size and shape and the specific arrangement of spines on the proboscis and body.

The short body, made up largely of an anterior expansion with a truncated rear-body is roughly comma-shaped. Spines in both sexes are continued into the rear quarter of the body dorsally and terminally on the lower side; in the male and female the end of the body is spined. The proboscis is armed with



FIGS. 17 – 18. *Corynosoma semerme ex. E. barbatus.* 17. *Entire worm, male.* 18. *Detail of proboscis.*

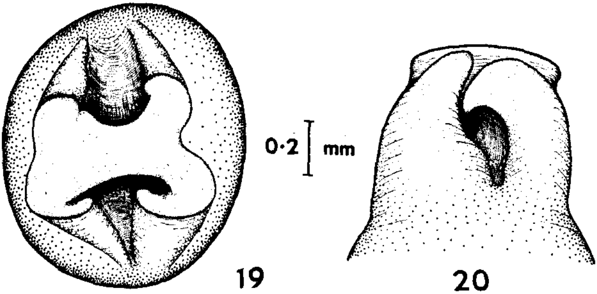
19 rows of spines. These are smallest in the first two rows (0.025 mm.), the remainder being uniform in size (0.062 mm.). The roots of these spines are never greater than about half the length of the free portion. The proboscis itself is distinctly more uniform in diameter than is the case with *C. strumosum* and measures about 0.7 by 0.25 mm. Organs are essentially similar to those of *C. strumosum* but more compactly arranged. The testes are laterally colinear, the lemnisci and proboscis sheath lie dorsally, and extend somewhat posterior, to them.

Cestodes

Cestodes require more careful technique in preserving and fixing than collections of this kind provide. For this reason, although considerable material has come to hand, all of it is in too poor condition to permit with certainty diagnosis though it is identifiable beyond doubt as falling within the genus *Diphyllobothrium*. All specimens are much contracted and in most decomposition has occurred to a lesser or greater extent. The collection is made up largely of broken strobila, some with the scolices included. Several specimens are complete animals; these are made up of more than 75 segments and in their contracted condition measure about 25 by 6.0 mm. The eggs in balsam mounts measure about 0.053 by 0.035 mm.; there was no significant variation from this size.

In most cases three to four uterine loops may be noted but occasionally there are as many as seven. In all cases the uterus lies definitely behind the cirrus sac, the uterine pore lying ventral to about the left rear margin of the sac.

The bothria are deep, separated by a frontal ridge, but reaching the anterior limit of the scolex in lateral perspective and may be traced to the posterior



Figs. 19 – 20. Diphyllobothrium sp. ex E. barbatus. 19. En face view. 20. Scolex, ventral view.

margin of the scolex. The scolex overlies the anterior part of the first segment behind. In heavily contracted specimens the scolex was roughly triangular in shape; isolated scolices were rectangular.

The differences in shape of the scolex and in the number of uterine coils would, by some standards, require species distinction. Stunkard and Schoenborn (12) have included these characteristics under *D. lanceolatum* and its synonyms, *D. schistochilos* and *D. coniceps*. Until it is possible to examine fresh material a diagnosis of this material as *D. lanceolatum* is indicated, and all distinguishable details conform.

The measurements do not disagree with the range listed for *D. lanceolatum*. The testes occupy a single medullary layer. The cirrus sac has a maximum diameter of 0.12 mm. with a relatively long, usually protruded cirrus; the double ovary has entire margins.

Further accurate details are not distinguishable.

The proglottids are longer than wide (1.8 by 2.4 mm. or wider than long (3.6 by 1.6 mm.). In a complete and highly compressed animal the proglottids were widest at about half the total length and were 0.4 mm. long and 5.5 mm. wide; the subterminal segment was 0.6 mm. long and 1.0 mm. wide.

Cestodes of this genus were recovered in *Erignathus barbatus* from Cape Smith, N.W.T., Cape Dorset, Baffin Island, and Wolstenholme, Que., and in *Phoca groenlandica* from Lake Harbour and Clyde River, Baffin Island.

The following host-parasite list summarizes our definite records in Canada to date:

Delphinapterus leucas (White Whale)
- *Anisakis simplex*
- *Anisakis* sp. inq.
- *Corynosoma strumosum*

Erignathus barbatus
- *Porrocaecum decipiens*
- *Corynosoma semerme*
- *C. strumosum*
- *Diphyllobothrium* sp.

Phoca vitulina (Harbour or Jar Seal)
- *Contracaecum osculatum*
- *Phocascaris netsiki*

Phoca groenlandica (Harp or Greenland Seal)
- *Porrocaecum decipiens*
- *Diphyllobothrium* sp.

Phoca hispida (Ringed Seal)
- *Contracaecum osculatum*
- *Corynosoma strumosum*
- *Phocascaris netsiki*

Of these, three records are new, the occurrence of *C. strumosum* in *D. leucas*, of *P. netsiki* in *P. hispida* and of *C. semerme* in *E. barbatus*.

Acknowledgments

The active co-operation of the Commissioner and men of the Royal Canadian Mounted Police, of the Fur Trade Commissioner and traders of the Hudson's Bay Company and of Dr. Vladykov and his colleagues was invaluable in collecting the material upon which this paper is based. The author is also keenly appreciative of the privileges extended by the Administration of the Northwest Territories associated with his appointment to the 1939 Eastern Arctic Patrol, which allowed personal collecting in Ellesmere, Baffin, Somerset and Southampton Islands and the western coast of Hudson Bay from the R.M.S. "Nascopie".

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THE DISTRIBUTION OF THE RED AND YELLOW COLOURS OF THE MUSCLE TISSUE OF BRITISH COLUMBIA CANNED SPRING SALMON AROUND THE MEANS OF THE INDIVIDUAL DISTRIBUTIONS OCCURRING IN SMALL, ARBITRARILY CHOSEN TIME INTERVALS[1]

BY F. CHARNLEY[2] AND LAURA M. HARCUS[3]

Abstract

The distributions of the red and yellow colours of the cooked muscle tissue of British Columbia spring salmon *(Oncorhynchus Tschawytscha)* around the means of the individual distributions occurring in small, arbitrarily chosen time intervals are composite distributions each consisting of two component normal distributions, thus indicating that there are two, and only two, varieties of this species when the salmon are classified on the basis of these two quality characteristics. The proportions of the pale and red varieties are very nearly 1 : 3 so that when sampling fluctuations are taken into account the data are in complete agreement with the hypothesis that the true proportions are, respectively, ¼ and ¾.

In an investigation of the functional forms of the distributions of the red and yellow colours of the muscle tissue of British Columbia canned salmon in connection with the grading of these characteristics, it was found that the distribution of the red colour of canned spring salmon *(Oncorhynchus Tschawytscha)* is markedly bimodal. The general shape of the distribution suggested that it was a composite distribution consisting of two component normal distributions with the red component forming considerably more than half the total population. If this hypothesis is correct and the species is homogeneous biologically, the data would indicate that the pale and red colours in this case are alternative Mendelian characters.

Before an answer to this last question can be undertaken, however, it is necessary to show that the distribution of the red colour in this species is in fact a mixture of two populations. Accordingly, the object of the investigation reported in this paper was to test the hypothesis as regards the functional form of the red colour and also to investigate that of the yellow colour of this species by fitting the experimental data with composite distributions made up of two component normal distributions.

Selection and Tabulation of Data

The data employed in the investigation were taken from the records of routine examinations of British Columbia canned spring salmon for the 1938 season and consist of the colour measurements made during these

[1] *Manuscript received June 3, 1940.*
Contribution from the Canned Salmon Inspection Laboratory, Department of Fisheries, Vancouver, B.C.
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examinations. The measurements were carried out by means of an Armstrong Colorimeter, an instrument (1) that utilizes the principle of matching the colour of the sample against coloured glasses. The intensity of colour is accordingly expressed in red and yellow Lovibond units.

In tabulating the original data preparatory to investigating the distributions of the red and yellow colours of the cooked muscle tissue of this species it was necessary to eliminate, as far as possible, three types of variations in the results. These were: (i) Variations arising from the fact that a number of companies pack only the red variety of this species, or, at least, only those salmon which, as far as can be determined, will definitely show red muscle tissue when cooked. (ii) Variations arising through geographical effects. For example, sockeye salmon caught in the northern areas in British Columbia are somewhat less intensely coloured than salmon caught in the southern areas, or in the Fraser River. The same seems to be true also of spring salmon. (iii) Variations resulting from seasonal effects.

Variations due to the first of these factors were eliminated by making careful inquiries of the companies who, as a result of preliminary inquiries, were believed to pack a certain proportion of, or all, unselected spring salmon. This information gave the canneries, in the area chosen, that had packed only unselected spring salmon during the 1938 season.

Variations resulting from the second factor were eliminated, as far as possible, by restricting the samples included in the investigation to those from the canneries in the area north of Knight Inlet that packed unselected samples during the 1938 season.

TABLE I

DISTRIBUTIONS OF THE RED COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

Red colour, Lovibond units	Interval No.																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.5	1	1	2	2	1			1	2	1							2
2.0	5	1	1	7	5	4	2	1	2	3	3	3	4	4	1		2
2.5		2	2	1	2	1	1	1	2	2	5	1	1	2	1		4
3.0	2	1	2				1	2		4	5	1	3	2	1	4	3
3.5	4	1			2	1	2	1	1	2	3	2	4	1	2		2
4.0	6	2	5	4	3	1		4	2	2	6	3	1	2	2		4
4.5	8	2	6	9	1	3	8	2	4	13	12	2	5	2		2	2
5.0	4	4	6	2	4	3	7	6	6	5	10	5	4	3			
5.5	3		1	5	1	3	1	2	2	10	1	2	2	1			3
6.0	1		1	2		1		2	3	2		1					
6.5											1			1			1
7.0										1							
7.5										1							
Total	34	14	26	32	19	17	22	22	24	46	46	20	24	18	7	6	23

TABLE II

DISTRIBUTIONS OF THE YELLOW COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

Yellow colour, Lovibond units	Interval No.																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.5	2	1				1			1								
2.0	3	1	1	3	3	1	3	1	2	2	1	1	2	3		1	3
2.5	6	2	6	8	5	5	2	4	4	6	13	5	10	6	4	1	9
3.0	12	5	8	9	3	1	6	8	7	17	10	4	5	6	2	4	5
3.5	8	2	4	5	2	6	7	4	5	11	10	4	4	1	1		3
4.0	1	2	2	5	5	1	2	4	3	5	12	5	2	2			2
4.5	2	1	5	2	1	2	2	1	2	3		1	1				1
5.0										2							
Total	34	14	26	32	19	17	22	22	24	46	46	20	24	18	7	6	23

Elimination of the variations arising from the third factor, however, was somewhat more difficult, because the mean intensities of the red and yellow colours of the muscle tissue of canned spring salmon show considerable seasonal trend. Since the distribution required is the distribution of the red or yellow colour around the line of seasonal trend and the absolute values of the means were of no particular interest, a simple method of combining the data evidently was to adjust the individual distributions occurring in small arbitrarily chosen time intervals to some convenient mean also arbitrarily chosen. For convenience in tabulating, the data were grouped in five-day intervals, the samples in any one time-interval thus being assumed to be derived from salmon of substantially the same biological condition. Time intervals were in all cases determined by the code marks.

Tables I and II show the distributions of the red and yellow colours of the samples of canned spring salmon when the samples are grouped in five-day intervals, while the trends in the means of the distributions given in Tables I and II are illustrated graphically in Fig. 1. From an inspection of the trends

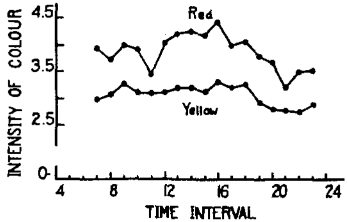

FIG. 1. *Curves showing seasonal trends in means of red and yellow colours of the muscle tissue of canned spring salmon corresponding to the data of Tables I and II.*

in Fig. 1 it will be observed that it is not necessary in all cases to shift the individual distributions to the arbitrary mean, since the distributions can be readily arranged into groups of approximately the same mean intensity of colour. A number of the means of the red colour lie on, or close to, the intensity 4.2. For convenience this was accordingly chosen as the arbitrary mean of the red colour.

To shift the remaining groups to this arbitrary mean we proceed as follows: Let h = grouping interval, d = shift in mean $= m_0 - m_i$, where m_i is the mean of the distribution that it is required to shift to the mean m_0. Then for $d < h$ the proportion of frequencies remaining in any one interval is $\frac{h - d}{h}$. The proportion of frequencies in the interval that are to be moved to the next or some succeeding interval is therefore $\frac{1 - h - d}{h} = \frac{d}{h}$. These formulae evidently apply also when the absolute value of d is greater than h, since, when the distribution is moved an integral number of intervals, its frequencies remain unchanged. To check the calculation the mean of the new distribution is calculated and compared with the arbitrary mean. An illustration of the calculations is shown in Table III.

TABLE III

DETAILS OF CALCULATIONS FOR INTERVAL No. 7 OF TABLE I ILLUSTRATING METHOD OF SHIFTING MEANS OF INDIVIDUAL DISTRIBUTIONS TO ARBITRARY MEAN.

X = intensity of red colour; f = frequency in original distribution; f_{i+1} = frequencies moved to next interval; f_i = frequencies remaining in interval; F = frequency in new distribution.

X	f	f_{i+1}	f_i	F	x	Fx
1.5	1	—	0.48	0.48	−5	− 2.40
2.0	5	0.52	2.40	2.92	−4	−11.68
2.5	—	2.60	—	2.60	−3	− 7.80
3.0	2	—	0.96	0.96	−2	− 1.92
3.5	4	1.04	1.92	2.96	−1	− 2.96
4.0	6	2.08	2.88	4.96	0	—
4.5	8	3.12	3.84	6.96	1	6.96
5.0	4	4.16	1.92	6.08	2	12.16
5.5	3	2.08	1.44	3.52	3	10.56
6.0	1	1.56	0.48	2.04	4	8.16
6.5	—	0.52	—	0.52	5	2.60
Total	34			34.00		13.68

d = 0.26; $h - d$ = 0.24. Distance from arbitrary origin = 0.402. Mean = 4.201.

In combining the data of Table 1 the distributions in intervals No. 13, 14, and 15 were assumed to have the same mean, namely 4.2, while the pairs of intervals 9 and 10, 17 and 18, and 22 and 23 have approximately the means 3.97, 4.04, and 3.51 respectively. Similarly in the case of the yellow colour the mean of each of the intervals 13 and 14, namely 3.2, was taken as the arbitrary origin, while intervals 10, 11, 12, and 15 were assumed to have the same mean 3.14, intervals 16, 17, and 18, the mean 3.25, and intervals 20, 21, and 22, a mean of 2.78.

Method of Fitting Combined Distributions

Table IV shows the combined or total distributions of the red and yellow colours derived from Tables I and II by combining the distributions occurring in the individual intervals as described in the preceding paragraphs. The general appearance of the distributions of the red and yellow colours of the muscle tissue of canned spring salmon when fewer intervals are combined is shown graphically in Figs. 2 and 3. In all instances, it will be observed, there is evidence that the distribution of the red colour is bimodal. The distribution of the yellow colour, however, is apparently unimodal. In the combined distribution of the red colour given in Table IV the dispersion of the measures around the two modes suggests that the distribution is a mixture of two component normal distributions. Since one of the components is present in smaller quantity than the other and very probably differs in standard deviation from the other, the data correspond to the general case of two component normal distributions.

TABLE IV

COMBINED DISTRIBUTIONS OF RED AND YELLOW COLOURS OF CANNED SPRING SALMON DERIVED FROM THE DATA OF TABLES I AND II

X = colour, F = frequency

Red colour				Yellow colour	
X	F	X	F	X	F
1.0	0.48	5.0	78.28	1.5	4.16
1.5	7.64	5.5	45.94	2.0	25.86
2.0	27.02	6.0	22.66	2.5	79.86
2.5	36.20	6.5	5.30	3.0	110.66
3.0	28.80	7.0	2.94	3.5	89.72
3.5	29.96	7.5	0.90	4.0	57.70
4.0	43.22			4.5	26.72
4.5	70.66			5.0	5.32
Total			400		400

The equations required in solving for the parameters of the component normal distributions in this case are readily derived from the characteristic function (2), namely,

$$\phi(t) = l_1 e^{a_1 t + b_1^2 \frac{t^2}{2}} + l_2 e^{a_2 t + b_2^2 \frac{t^2}{2}},$$

by taking successive derivatives of this function with respect to t. On putting $t = 0$ in the results we easily obtain the successive moments of the distribution in terms of the unknown proportions l_1 and l_2, the means a_1 and a_2, and the standard deviations b_1 and b_2 of the component normal distributions. The required moments are as follows:

(1) $\mu_0 = 1 = l_1 + l_2$

(2) $\mu_1 = l_1 a_1 + l_2 a_2$

(3) $\mu_2 = l_1(a_1^2 + b_1^2) + l_2(a_2^2 + b_2^2)$

(4) $\mu_3 = l_1 a_1^3 + 3 l_1 a_1 b_1^2 + l_2 a_2^3 + 3 l_2 a_2 b_2^2$

$$(5) \quad \mu_4 = l_1(a_1^4 + 6a_1^2b_1^2 + 3b_1^4) + l_2(a_2^4 + 6a_2^2b_2^2 + 3b_2^4)$$

$$(6) \quad \mu_5 = l_1(15b_1^4a_1 + 10b_1^2a_1^3 + a_1^5) + l_2(15b_2^4a_2 + 10b_2^2a_2^3 + a_2^5),$$

in which μ_0, μ_1, etc. are the various moments of the combined distribution.

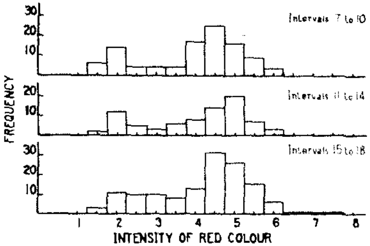

FIG. 2. *Distributions of intensity of the red colour obtained on combining the individual distributions in four successive time intervals.*

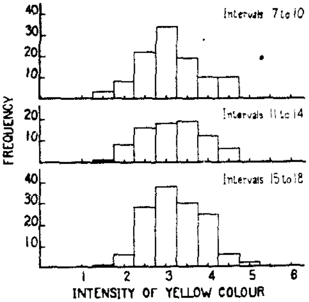

FIG. 3. *Distributions of intensity of the yellow colour obtained on combining the individual distributions in four successive time intervals.*

These equations, which were first derived by Pearson, permit a solution of the general case of a distribution consisting of two component normal distributions. An examination of the combined distribution of the red colour given in Table IV, however, shows that at least half, and possibly more than half, of the distribution in the upper part of the range, that is, a large portion of the red variety in the distribution, may be considered homogeneous. Hence, instead of solving Equations (1) to (6) by the laborious procedure of elimination and solution of the resulting nonic, we may employ the relatively rapid method described by Pearson (5).

Let d be the distance of the stump of the tail from the mean of the truncated portion and Σ the standard deviation of the truncated portion about its mean. Also let ν_1^1, ν_2^1 be the first and second moments of the truncated portion around an arbitrary origin x_0 units distant from the stump, $\mu_2 = \sigma^2$ the second moment of the normal distribution around its mean, x the distance from the stump to the mean of the curve, $\bar{x}$ the distance of the mean of the truncated portion to the mean of the curve, n the area of the truncated portion, N the whole population, m_0, m_1, etc., the various normal moment functions, and i the grouping interval. Then as shown by Pearson

$$d = x_0 + \nu_1^1 i = \bar{x} + x$$

$$\Sigma^2 = \nu_2 i^2 = \{\nu_2^1 - (\nu_1^1)^2\} i^2$$

$$\frac{n}{N} = \tfrac{1}{2} + m_0 \qquad (7)$$

$$n\bar{x} = N\sigma\left(\frac{1}{\sqrt{2\pi}} - m_1\right) \qquad (8)$$

$$n\mu_2 = N\sigma^2(\tfrac{1}{2} + m_2) \qquad (9)$$

$$\frac{d}{\sigma} = \frac{\dfrac{1}{\sqrt{2\pi}} - m_1 + \dfrac{x}{\sigma}(\tfrac{1}{2} + m_0)}{\tfrac{1}{2} + m_0} \qquad (10)$$

$$\frac{\Sigma^2}{d^2} = \frac{(\tfrac{1}{2} + m_2)(\tfrac{1}{2} + m_0) - \left(\dfrac{1}{\sqrt{2\pi}} - m_1\right)^2}{\left\{\dfrac{1}{\sqrt{2\pi}} - m_1 + \dfrac{x}{\sigma}(\tfrac{1}{2} + m_0)\right\}^2} \qquad (11)$$

The graph of the combined distribution of the red colour given in Table IV suggests that it is safe to assume the truncated portion bounded by the stump 3.75 red units as being very closely homogeneous. Accordingly, the first and second raw moments ν_1^1 and ν_2^1 (Table V) are respectively 2.8752 and 10.0940, n is 269.90, Σ, 1.8272 and d, 1.1876. Hence $\frac{\Sigma^2}{d^2} = \psi_1 = 0.3239$. By trial it is found on applying Equation (11) that this value of ψ_1 corresponds

TABLE V

STATISTICAL DATA USED IN CALCULATING THE MEANS AND STANDARD DEVIATIONS OF THE COMPONENT DISTRIBUTIONS IN THE COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS

Distribution of red colour

Arbitrary origin = 4.0; μ_1^1 = 0.4020 half units; μ_2 = 6.2229 half units ^{2}; μ_1 = 0; μ_2 = 1.5557 units; Total number of measures = 400.

Truncated portion of distribution of red colour

Arbitrary origin = 3.5; stump = 3.75 red units; n = 269.90; ν_1^1 = 2.8752 half units; ν_2 = 1.8272 half units^2; d = 1.1876; Σ^2 = 0.4568 units.

Distribution of yellow colour

Arbitrary origin = 3.0; μ_1^1 = 0.4063 half units; μ_2 = 2.0021 half units^2; μ_3 = 0.5283 half units^3; μ_4 = 10.5560 half units.^{4}

to a value of $x' \left(= \frac{x}{\sigma}\right)$ lying between 1.2 and 1.3. On calculating the value of ψ_1 corresponding to $x' = 1.4$ and applying Stirling's interpolation formula or Formula (iii) in Pearson's "Tables for Statisticians and Biometricians" to the three values 1.2, 1.3, and 1.4 of x' and the corresponding values of ψ_1, we find that x' corresponding to the above value of ψ_1, namely 0.3239, is 1.2965. Application of the first two terms of the interpolation formula to the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the same three values of x' then gives the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the calculated value of x', that is, to $\psi_1 = 0.3239$. The substitution of these values together with the experimental value of d in Equation (10) gives $\frac{d}{\sigma} = 1.4872$, and hence $\sigma = b_1 = 0.7985$. Consequently $x = x'\sigma = 1.0353$; $a_1 = 3.75 + 1.0353 - 4.201 = 0.5843$; $\frac{n}{N} = \frac{1}{2} + m_0 = 0.9026$, and $N =$ 299.027.

To find the mean and standard deviation of the other component distribution, that is, of the pale variety of spring salmon, we substitute the parameters of the red component in Equations (1), (2), and (3). The value of l_1 is 0.7476, so that l_2 is 0.2524. The first moment of the combined distribution μ_1 is zero and the second moment $\mu_2 = 1.557$. Equations (2) and (3) therefore give $a_2 = -1.7304$ and $b_2 = 0.5188$ or, referred to the arbitrary mean, $a_2 = 2.4706$.

As mentioned above, the combined distribution of the yellow colour is unimodal and from an examination of the plotted data appears to be approximately normal. The values of the statistics k and β_2 of this distribution, however, suggest that this distribution also has been drawn from a non-normal population. The values of k and β_2 are 0.1865 and 2.6334, respectively. Assuming that the distribution of the yellow colour is approximately normal we have $\sigma_k = \sqrt{\frac{6}{n}} = 0.1225$ and $\sigma_{\beta_2} = \sqrt{\frac{24}{n}} = 0.2450$, so that the calculated values deviate approximately 1.5 standard deviations from the expected values in each case. The values of k and β_2, however, also deviate in the directions that would be expected if the combined distribution of the yellow colour consists of two component distributions similar to those in the combined distribution of the red colour but differing much less in their means. The chances of drawing two simultaneous values of k and β_2 from a normal population each deviating 1.5 standard deviations from the expected value is about 1 chance in 60 and the probability that the two values will deviate in this particular direction is one-quarter of this. Hence there is a strong indication that the distribution of the yellow colour is also a mixture of two component populations corresponding to those in the distribution of the red colour.

In view of the close chemical relationship that exists between chlorophyll and the blood pigments and also the close biological relationship that exists

between the red and yellow colours of canned salmon as indicated by the relatively high correlation between the red and yellow colours, it seems safe to assume that the proportions l_1 and l_2 of the two varieties in the combined distribution of the yellow colour will be the same as those found for the distribution of the red colour. With this assumption the parameters of the component distributions of the yellow colour may then be calculated by means of Equations (2) to (5) from the first four moments of the distribution. The required moments are $\mu_1 = 0$, $\mu_2 = 2.0021$, $\mu_3 = 0.5283$ and $\mu_4 = 10.5560$. Substitution of these values of the moments and the values of l_1 and l_2, namely 0.7476 and 0.2524 respectively, in Equations (2) to (5) thus gives four equations in the unknown a_1, a_2, b_1, and b_2, from which by the elimination of a_2, b_1, and b_2 we obtain the sextic $a_1^6 + 0.05497a_1^3 - 0.05845a_1^2 - 0.00125 = 0$.

The application of Descartes' rule of signs shows immediately that this equation has only one real positive root. Putting $a_1 = 0$ and $a_1 = 1$ in this equation we find that $F(0) = -0.002036$ and $F(1) = 1.0032$ and hence the root lies between 0 and 1. By applying Horner's method the positive root is then found to be $a_1 = 0.4474$.

Since the mean of the combined distribution measured from the arbitrary origin 3.0 was in terms of half the original units as were also the moments, the resulting values of the parameters are also in half units. The mean of the red variety in half units was 0.4062. Accordingly, the mean of the red variety is 3.2031 + 0.2237 = 3.4268 yellow units. The value of a_2 follows at once and is $a_2 = -1.2552$, that is, the mean of the pale variety is 2.5755 yellow units. Similarly, the corresponding standard deviations are $b_1 = 1.2985$, $b_2 = 0.8771$, so that when expressed in whole units the standard deviations of the yellow colours of the red and pale varieties are 0.6493 and 0.4385, respectively.

Test of Hypothesis

To test the hypothesis as regards the functional forms of the two combined distributions we compare the theoretical distributions with the experimental distributions given in Table IV. This comparison may be effected either graphically or by means of the χ^2 test or by both methods.

Table VI shows the calculated or theoretical values of the ordinates corresponding to given values of the intensity of colour for each of the experimental distributions given in Table IV. These data are illustrated graphically in Figs. 4 and 5. From an inspection of these figures it will be observed that the theoretical distributions describe with a high degree of accuracy the experimental data.

This conclusion is also confirmed by the χ^2 test. Tables VII and VIII show the observed and theoretical frequencies falling into the various classes in the combined distributions of the red and yellow colours. The number of classes and the value of χ^2 for the combined distribution of the red colour are respectively 11 and 2.080, and the corresponding figures for the combined

TABLE VI

ORDINATES OF THE COMPONENT NORMAL DISTRIBUTIONS AND OF THE RESULTING COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS CORRESPONDING TO THE DATA OF TABLE IV

Colour	Red			Yellow		
	Pale variety	Red variety	Combined	Pale variety	Red variety	Combined
1.0	0.72		0.72	0.07	0.09	0.16
1.5	6.77		6.77	2.30	1.17	3.47
2.0	26.08	0.19	26.27	19.54	8.48	28.02
2.5	38.79	1.25	40.04	45.29	33.88	79.17
3.0	23.54	6.29	29.83	29.20	74.73	103.93
3.5	5.51	21.08	26.59	5.16	91.34	96.50
4.0	0.52	46.34	46.86	0.25	62.53	62.78
4.5		70.41	70.41		23.65	23.65
5.0		72.38	72.38		4.95	4.95
5.5		50.51	50.51		0.58	0.58
6.0		23.59	23.59			
6.5		7.69	7.69			
7.0		1.63	1.63			
7.5		0.24	0.24			

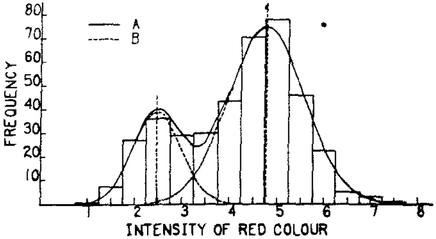

FIG. 4. *Graphical illustration of the combined distribution of the intensity of the red colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.*

distribution of the yellow colour are 8 and 2.257. Since the observed data in this case were used in calculating the values of the parameters in the theoretical distribution, the number of classes (7) must be diminished by six in each case in computing the number of degrees of freedom, since this is the number of conditions employed in calculating the values of the parameters. The number of degrees of freedom of the distributions of the red and yellow colours are accordingly 5 and 2 respectively. Reference to Fisher's table (3) shows that both these values of χ^2 might very easily have arisen through sampling fluctuation. The probability associated with the value 2.080 is approximately $P = 0.85$, while that associated with the value

2.257 is about $P = 0.50$. There is thus no reason to suspect the hypothesis tested and the theoretical distribution must, therefore, be regarded as fitting very closely the experimental data.

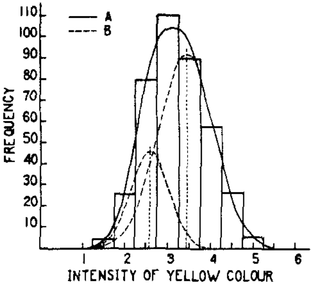

FIG. 5. *Graphical illustration of the combined distribution of the intensity of the yellow colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.*

TABLE VII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE RED COLOUR

Colour	Obs'd f $(m + x)$	Calc'd f (m)	x	$\frac{x^2}{m}$
0.5, 1.0, 1.5	8.12	8.32	−0.20	0.005
2.0	27.02	25.73	1.29	0.065
2.5	36.20	38.74	−2.54	0.166
3.0	28.80	29.62	−0.82	0.023
3.5	29.96	27.00	2.96	0.324
4.0	43.22	46.68	−3.46	0.256
4.5	70.66	69.12	1.54	0.034
5.0	78.28	70.96	7.32	0.755
5.5	45.94	49.88	−3.94	0.311
6.0	22.66	23.98	−1.32	0.073
6.5, 7.0, 7.5, 8.0	9.14	9.96	−0.82	0.068
Total	400.00	399.99		2.080

Concluding Remarks

Sheppard's corrections for grouping, it will be observed, were not applied in the foregoing calculations, but instead, the raw moments were substituted directly into the equations. Shewhart (7) gives a number of reasons why it may not be advisable in all cases to apply such corrections. In the present

TABLE VIII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE YELLOW COLOUR

Colour	Obs'd f $(m+x)$	Calc'd f (m)	x	$\frac{x^2}{m}$
1.0, 1.5	4.16	4.47	−0.31	0.021
2.0	25.86	29.08	−3.22	0.356
2.5	79.86	76.97	2.89	0.108
3.0	110.66	101.61	9.05	0.806
3.5	89.72	95.00	−5.28	0.293
4.0	57.70	62.24	−4.54	0.331
4.5	26.72	24.42	2.30	0.217
5.0, 5.5, 6.0	5.32	6.20	−0.88	0.125
Total	400.00	399.99		2.257

instance the most cogent reason for not applying the corrections would appear to be the relatively small sample size (269.9) employed in calculating the second moment of the truncated portion of the distribution of the red colour. If h is the grouping interval, Sheppard's correction for the second moment is $-\frac{h^2}{12}$. In this instance, however, the standard deviation of the second moment is (4, 6).

$$\sigma_{\mu_2} = \sqrt{\frac{\mu_4 - \mu_2^2}{n}},$$

where n is the sample size. The values of μ_4 and μ_2 of the truncated part expressed in half units were 11.4430 and 1.8272 respectively. Hence, in half units $\sigma_{\mu_2} = 0.1733$ or $\sigma_{\mu_2} = 0.04332$ red units. Sheppard's correction, namely, 0.02083, is thus less than half the standard error of the second moment, so that it would be a useless refinement to apply the correction in this case.

Another point that should be noted in the above calculations is the approximation employed in combining the individual distributions in the various time intervals. The means of the individual distributions have been used as estimates of the true means of the red and yellow colours corresponding to the respective time intervals. This procedure will introduce slight errors into the final results owing to sampling fluctuations in the means. Here again, however, the simpler procedure would seem to be preferable in this case to the questionable refinement of fitting the trends with meaningless third or fourth degree power series.

Finally, it is interesting to note that the difference in the means of the red colour for the two varieties is 4.7853 − 2.4706 = 2.3147 and the difference in the means of the yellow colour, 3.4268 − 2.5755 = 0.8513. The absolute values of the means, of course, are arbitrary. Also, the two varieties of spring salmon occur very nearly in the proportions of 1 to 3, that is, very nearly one-

quarter of all the samples consist of the pale variety while the remaining three-quarters is made up of the red variety of this species.

The value 0.7476, it will be observed, is in complete accord with the hypothesis that the true proportion is 0.7500. For, if a sample of 400 were drawn from a population in which the true proportion of the red variety was 0.7500, the standard deviation of the observed proportion would be

$$\sigma_p = \sqrt{\frac{pq}{n}} = 0.02165.$$

Consequently, the observed value 0.7476 differs only slightly more than one-tenth standard deviation from the hypothetical value 0.7500 and there are accordingly no grounds for concluding that the true proportion is not 0.7500.

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PARASITES OF FRESHWATER FISH

III. FURTHER STUDIES ON THE INTERNAL TREMATODES OF FISH IN THE CENTRAL ST. LAWRENCE WATERSHED[1]

By M. J. Miller[2]

Abstract

Three new species of internal trematodes, *Plagioporus serratus* sp. nov., *Phyllodistomum lysteri* sp. nov., and *Parastiotrema ottawanensis* gen. et sp. nov., are described from Canadian freshwater fish. *Plagioporus serotinus* Stafford, 1904, is redescribed, and the genus *Caudotestis* Yamaguti, 1934, is reduced to synonymity with *Plagioporus* Stafford, 1904. *Anallocreadium pearsei* Hunter and Bangham, 1932, is considered synonymous with *A. armatum* McCallum, 1895. The genus *Bunoderina* Miller, 1936, is reduced to synonymity with *Bunodera* Railliet, 1896.

Introduction

This paper, the third in a series on the parasites of freshwater fish in Canada, presents the results of the survey that has been continued since the first paper (6) appeared; it deals with non-commercial as well as commercial fish. All the fish were taken from the Ottawa River near its confluence with the St. Lawrence River, at Ste. Anne de Bellevue, Quebec.

Species Recovered

FAMILY ALLOCREADIIDAE

Genus *Plagioporus* Stafford, 1904

Synonyms: *Lebouria* Nicoll, 1909,

Caudotestis Yamaguti, 1934.

Stafford created the genus *Plagioporus* to include *P. serotinus*, a trematode he recovered from the intestines of *Moxostomata macrolepidotum* (red horse sucker). Nicoll (9) erected the genus *Lebouria* for his species *L. idonea* and included in the genus a trematode described by Linton from *Bairdiella chrysura* and named by Nicoll *L. abducta*. Price (12) pointed out that *Lebouria* was congeneric with *Plagioporus* and, as the latter genus has priority, he transferred the species of *Lebouria* to the genus *Plagioporus*, thus reducing *Lebouria* to synonymity. The subgenus *Caudotestis* (Issaitschikow, 1928) was raised to generic rank by Yamaguti (16) to include those species of *Plagioporus* having the intestinal caeca terminating at the level of the testes, and the vitellaria usually not extending into the post-testicular body region. However, these two characters are not sufficiently well defined in the species of the two genera, which show all the intergradations. Thus, *P. cooperi* has the caeca terminating at the level of the testes, but the post-testicular vitellaria

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are numerous, and meet medially. It is apparent, therefore, that *Caudotestis*, as it is now defined, cannot be retained as a separate genus and must be considered as a synonym of *Plagioporus*.

Mueller (8) stated that Stafford's species *P. serotinus* could not be retained because it was not recognizable. However, the type specimen has been recovered. Furthermore, in the intestines of the red horse and common sucker have been found additional specimens which differ only in that they are of a larger size in the former host.

Plagioporus serotinus Stafford, 1904
(Figs. 2 and 3)

HOSTS: *Moxostoma aureolum* (red horse sucker)
Catostomus commersonii (common sucker)

The worms of this species are small, narrow forms, broadest in the middle and tapering gradually to both extremities. The cuticula is smooth. They measure from 1.0 to 1.4 mm. in length in the common sucker, whereas specimens from the red horse sucker measure up to 2.2 mm. in length. They are approximately one-fourth as broad as long. The oral sucker is terminal,

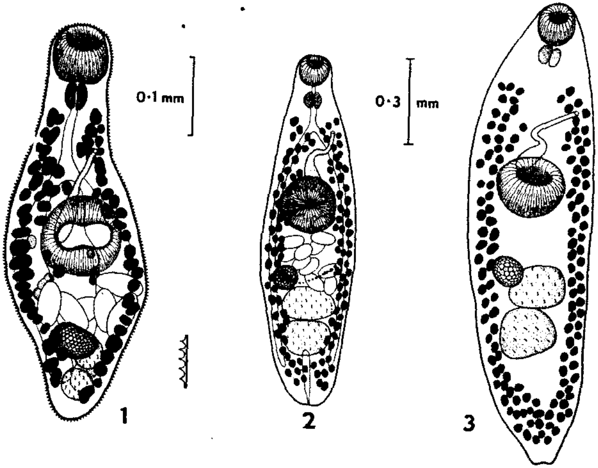


FIG. 1. *Plagioporus serratus, ventral view.* FIG. 2. *P. serotinus, ventral view, specimen from common sucker.* FIG. 3. *P. serotinus, ventral view, specimen from Stafford's collection from red horse sucker.*

subspherical, and measures from 0.08 to 0.14 mm. in diameter. The acetabulum is situated two-fifths of the body length from the anterior tip, and is approximately twice the diameter of the oral sucker. There is a short prepharynx and a well developed pharynx whose length is about half the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The intestinal caeca extend to within a short distance of the posterior extremity. The testes are in tandem arrangement, one immediately behind the other, in the middle of the posterior half of the body. Occasionally the anterior testis is displaced slightly to the left. The testes are smooth-margined and usually somewhat flattened in the antero-posterior axis. The ovary is spherical and is situated immediately anterior of the anterior testis, on the right side of the body. The vitellaria extend laterally from near the pharynx to within a short distance from the posterior tip; they meet in the post-testicular body space. The uterus extends anteriorly from the ovary to the genital pore, which is situated at the level of the intestinal bifurcation, on the left side of the body. The cirrus pouch overlaps the acetabulum one-quarter to one-half its diameter. The excretory bladder is small and sac-like. The eggs measure 0.07 to 0.09 mm. by 0.05 to 0.06 mm.

Plagioporus serratus sp. nov.
(Fig. 1)

HOST: *Hyodon tergisus* (moon-eye)

Members of this species occur in the gall bladder of their host. They are very small, somewhat spindle-shaped flukes, tapering rather abruptly at the posterior tip and narrowing out to a broad neck at the anterior end. The cuticula is characteristically extended to form conspicuous broad-based spines, which cover the entire body. Mature specimens measure from 0.41 to 0.51 mm. in length by 0.14 to 0.18 mm. in width at the widest point, which is usually the region of the acetabulum. The oral sucker is terminal, subspherical and measures from 0.076 to 0.080 mm. in diameter. The acetabulum is situated about the middle of the body. It is subspherical and somewhat larger than the oral sucker, measuring from 0.09 to 0.11 mm. in diameter. There is no obvious pre-pharynx. The pharynx is prominent and is about half as long as the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The broad intestinal crura extend to within a short distance of the posterior extremity. The testes are obliquely arranged near the posterior tip of the body; the posterior testis is on the mid-line and the anterior testis is immediately to the left of it. They are approximately spherical in outline with smooth edges, and measure from 0.042 to 0.046 mm. in diameter. The ovary is ovate in outline with the long axis in the lateral plane; it is larger than the testes and is situated on the mid-line partly overlapping the anterior testis. The vitellaria extend laterally from the posterior end of the pharynx to the posterior testis. The uterus passes anteriorly to the genital pore situated on the left side of the body slightly

anterior to the level of the intestinal bifurcation. The cirrus pouch overlaps the acetabulum to about half its diameter. The eggs are comparatively large, measuring 0.067 by 0.038 mm. There are usually from four to six eggs in the uterus.

Plagioporus serratus differs from all previously described species of this genus by its extremely small size, and the spinose extensions of its cuticula.

As far as can be determined eight species of *Plagioporus* have been reported from American fishes. Included in this number is *Plagioporus (Lebouria) abducta* (Nicoll, 1909), a parasite described by Linton (1904) from *Bairdiella chrysura*. However, *P. abducta* cannot be retained in the genus *Plagioporus*, as the median position of its genital pore violates the concepts of the genus. A key to the American species of *Plagioporus* is presented below.

1. Cuticula with large spinose extensions; small worms under 0.6 mm. in length..*P. serratus* sp. nov.
   Cuticula smooth, without spinose extensions......................2.
2. Body strongly fusiform; acetabulum broadly ovate with the long axis in the lateral plane, over twice the size of the oral sucker.
   P. fusiformis Price, 1934.
   Body not strongly fusiform; acetabulum more or less spherical, not over twice the diameter of the oral sucker........................3.
3. Vitellarian follicles numerous in post-testicular body region............4.
   Vitellarian follicles not numerous in post-testicular body region, extending just beyond the testes; testes near posterior tip of body.
   P. sinitsini Mueller, 1934.
4. Intestinal crura not extending posterior of the posterior testis.
   P. cooperia Hunter and Bangham
   Intestinal crura extending posterior of the posterior testis..........5.
5. Vitellarian follicles not extending anterior of the acetabulum.
   P. lepomis Dobrovolny, 1939.
   Vitellarian follicles extending anterior of the acetabulum..........6.
6. Vitellarian follicles anterior of the acetabulum for the most part confined laterally; acetabulum approximately twice the diameter of the oral sucker...............................*P. serotinus* Stafford, 1904.
   Vitellarian follicles anterior of the acetabulum meeting medially; acetabulum considerably less than twice the diameter of the oral sucker...............................*P. crassigula* Linton, 1911.

Anallocreadium armatum McCallum, 1895

(Fig. 10)

Synonym: *Anallocreadium pearsei* Hunter and Bangham, 1932.

HOSTS: *Ameiurus nebulosus* (bullhead).
Eupomotis gibbosus (sunfish).

This parasite was found rather commonly in the intestine of sunfish, but only five specimens were recovered from the bullhead. All specimens with the exception of one from the sunfish and two from the bullheads contained no eggs.

The mature specimens measure from 1.23 to 2.31 mm. in length by 0.5 to 0.8 mm. wide. They have rounded extremities with the posterior end broader. Spines can be seen on the cuticula of some specimens but only with difficulty. The oral sucker is subspherical. There is a well developed pre-pharynx, a pharynx which measures slightly less than half the diameter of the oral sucker, and a short oesophagus which forks about one-third of the distance between the pharynx and the acetabulum. The intestinal caeca extend well into the posterior portion of the body. The acetabulum is slightly larger than the oral sucker, and is situated in the second quarter of the body. The testes are in tandem arrangement in the third quarter of the body. they are broadly ovate to spherical in outline with smooth margins and may be smaller or larger than the acetabulum. The ovary is small, spherical in outline and situated on the right side of the body immediately anterior of the anterior testis. The vitellaria extend laterally from the posterior margin of the acetabulum to the posterior tip of the body filling in the post-testicular space. The uterus passes anterior from the ovary to the genital pore situated on the mid-line immediately anterior of the acetabulum. Measurements of an average specimen are as follows: length 2.06 mm., width 0.59 mm., oral sucker 0.22 mm. in diameter, acetabulum 0.25 mm. in diameter, ovary 0.13 mm. in diameter, anterior testis 0.24 by 0.31 mm., posterior testis 0.26 by 0.31 mm., egg 0.09 to 0.11 by 0.06 mm.

Hunter and Bangham (4) created the species *Anallocreadium pearsei* for a trematode in the intestine of *Aplodinotus grunniens.* Pearse (11) reported a species of *Anallocreadium* from the same host and from *Eupomotis gibbosus* which he called *A. armatum* but which Hunter and Bangham consider to be *A. pearsei.* The characters used by these authors to separate *A. pearsei* from *A. armatum* are the nature of the margin of the testes, and the comparative sizes of the suckers, of the pharynx, and acetabulum, and of the testes and acetabulum. According to them *A. pearsei* has the oral sucker only slightly smaller than the acetabulum, while *A. armatum* has an acetabulum twice the size of the oral sucker. They consider the testes to be always larger than the acetabulum in *A. pearsei* and usually smaller in *A. armatum.* They state the length of the pharynx to be about one-third the diameter of the acetabulum in *A. armatum* and one-half the diameter in *A. pearsei.* Finally, they consider the testes to have smooth margins in *A. armatum* and lobed margins in *A. pearsei.*

The specimens recovered in the present survey show characters of both species. The oral sucker is only slightly smaller than the acetabulum which is characteristic of *A. pearsei,* but the testes are smooth-margined, a characteristic of *A. armatum.* The length of the pharynx varies from one-third to one-half the diameter of the acetabulum, and the testes range in size from

smaller, to considerably larger than, the acetabulum. It appears, therefore, that there is but one valid species of *Anallocreadium*, and that *A. pearsei* must be considered a synonym of *A. armatum*.

Bunodera eucaliae (Miller, 1936)

Synonym: *Bunoderina eucaliae* Miller, 1936

HOST: *Eucalia inconstans* (stickleback).

This parasite, originally referred to a new genus by the author because of the character of its uterus which is tubular rather than sacculate, resembles *Bunodera sacculata* Van Cleave and Mueller (15) in so many characters that it seems more consistent to place it in the same genus. The tubular uterus in *B. eucaliae* probably points out the relationship of *Bunodera* and *Crepidostomum* and may indicate the direction of the evolutionary trend.

Three species of *Bunodera* have been reported from North America. They can be distinguished as follows:

1. Vitellaria extending laterally from anterior of the acetabulum to near the posterior tip of the body . . . . *B. luciopercae* Müller, 1776.
   Vitellaria extending laterally from anterior of the acetabulum to about the middle of the body length . . . . 2.
2. Vitellaria not extending to the level of the testes; acetabulum larger than oral sucker; mature forms with tubular uterus. . *B. eucaliae* Miller, 1936.
   Vitellaria extending to the level of the testes; acetabulum not larger than oral sucker; mature forms with sacculate uterus
   B. sacculata Van Cleave and Mueller, 1934.

FAMILY PLAGIORCHIDAE

Parastiotrema ottawanensis gen. et. sp. nov.

(Figs. 4 and 5)

HOST: *Ictalurus punctatus* (channel catfish)

Only three specimens of this small trematode were recovered from the intestine of the channel catfish. One of the specimens was contracted to such an extent that it could not be studied.

Members of this species are small muscular worms, pointed at the posterior tip and more or less rounded anteriorly. The middle part of the body is parallel-sided. The cuticula is covered with very fine spines which are more prominent anteriorly and gradually disappear toward the posterior part of the body. One of the specimens is longer and narrower than the other, measuring 0.72 by 0.22 mm.; the other measures 0.58 by 0.27 mm. The oral sucker is spherical to subspherical in outline; in one specimen it measures 0.092 and in the other 0.108 mm. in diameter. The acetabulum is approximately the same size as the oral sucker. It is situated in the first or second quarter of the body, depending on the state of contraction of the body. The pharynx is about half as long as the oral sucker. There is no apparent pre-

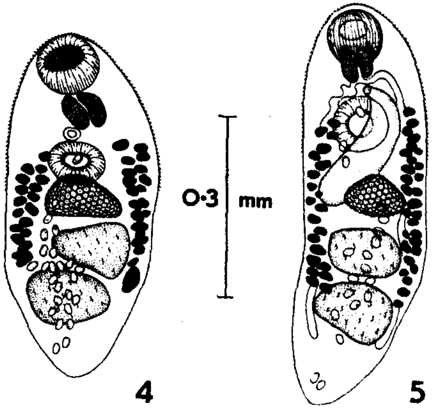


FIG. 4. *Parastiotrema ottawanensis, ventral view.* FIG. 5. *P. ottawanensis, dorsal view.*

pharynx nor does there appear to be an oesophagus. The intestinal crura lead to the posterior border of the posterior testis. The testes are comparatively large, with smooth or slightly irregular margins; they are roughly triangular in shape with the long axis in the lateral plane. They measure up to 0.08 mm. in width, and are in tandem arrangement with the anterior displaced slightly to the left. The ovary is situated between the anterior testis and the acetabulum. It is triangular in shape and somewhat smaller than the testes. The uterus passes back to the posterior tip of the body. The vitellaria extend laterally from the anterior margin of the acetabulum to the posterior margin of the posterior testis. The genital pore is situated on the mid-line immediately anterior of the acetabulum. The cirrus pouch is long and broad extending posteriorly to overlap the ovary. The eggs measure 0.025 by 0.017 mm.

The genus *Parastiotrema* appears to be closely related to *Astiotrema* Looss, 1900, and *Alloglossidium* Mueller, 1930. Like *Astiotrema* it has a very large cirrus pouch which differs, however, in that, unlike the cirrus sac in that species, it does not have the terminal end particularly thin. The large triangular shaped genital organs are quite distinct from those exhibited by the species of *Astiotrema* as is the character of the alimentary tract. It differs from *Alloglossidium* mainly in the character of the alimentary tract, and in the shape and size of the testes and ovary.

Generic Diagnosis

Plagiorchiinae; small parallel-sided forms, more or less pointed at the posterior extremity, and rounded at the anterior end. Cuticula with minute spines; alimentary tract with well developed oral sucker and pharynx, but with no apparent pre-pharynx or oesophagus. Testes and ovary large, roughly triangular in outline; testes in the posterior half of the body in tandem arrangement; ovary anterior of the anterior testis; uterus extending back to the posterior tip of the body. Cirrus sac very large and broad, extending back to the posterior margin of the ovary; vitellaria laterally arranged from the acetabulum to the testis; occurring in the intestine of fishes.

Alloglossidium geminus Mueller, 1930
(Fig. 9)

HOST: *Ameiurus nebulosus* (bullhead).

Specimens of this species are common in the intestine of the bullhead in this vicinity. They range from 0.68 to 1.27 mm. in length.

Alloglossidium corti Lamont, 1921
(Figs. 7 and 8)

HOST: *Ictalurus punctatus* (channel catfish).

This species was very commonly recovered from the intestine of the channel catfish. Mature specimens ranged from 0.53 to over 2.0 mm. in length. The smaller specimens have the genital organs and the acetabulum situated proportionately further back in the body, and the body is comparatively wider. The presence of intermediate forms, however, shows the two types to be of the same species. As pointed out by van Cleave and Mueller (15) this species can be separated from *A. geminus* by the character of the vitellaria which extend anterior of the acetabulum in *A. corti*, and not anterior of the anterior margin of the acetabulum in *A. geminus*.

FAMILY HETEROPHYIDAE

Cryptogonimus chyli Osborn, 1903
(Fig. 6)

HOSTS: *Ambloplites rupestris* (rock bass)
Micropterus dolomieu (small-mouth black bass)

Only a few specimens of this species were obtained from the above mentioned hosts.

FAMILY AZYGIIDAE

Azygia angusticauda Stafford, 1904

HOSTS: *Stizostedion vitreum* (doré)
Micropterus dolomieu (small mouth black bass)

Three specimens of this species were recovered, one from the intestine of the bass, and the other two from the intestine of the doré. The specimen

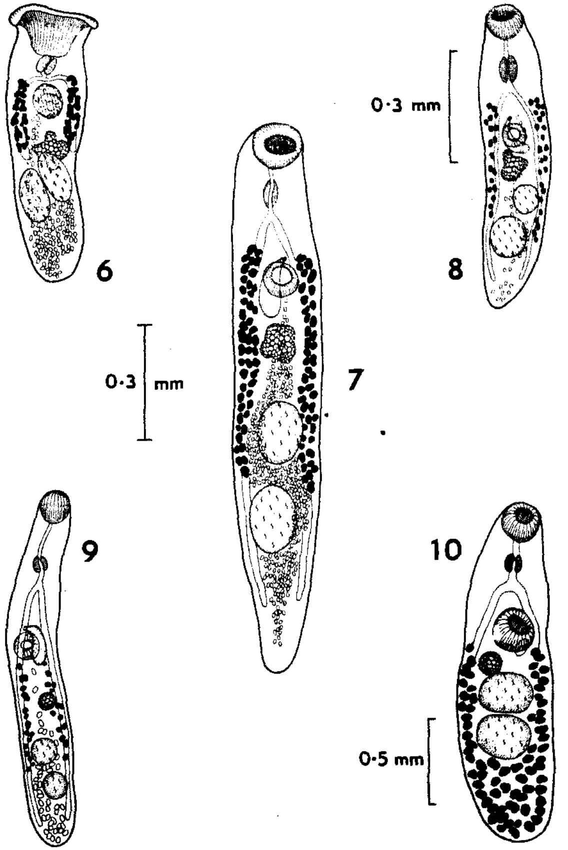


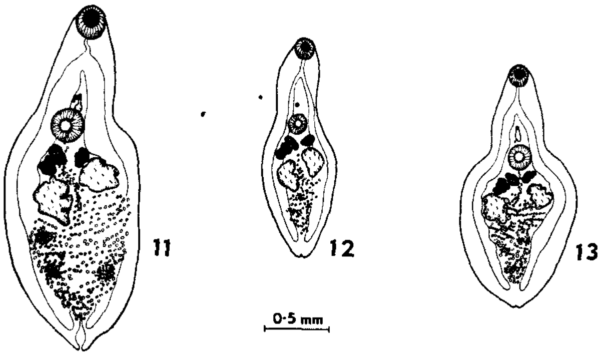
FIG. 6. *Cryptogonimus chyli, dorsal view.* FIGS. 7 AND 8. *Alloglossidium corti, ventral views.* FIG. 9. *A. geminus, dorsal view.* FIG. 10. *Anallocreadium armatum, dorsal view.*

from the bass measured slightly less than 2.0 mm. in length, while those from the doré measured 11 and 12 mm. respectively. Van Cleave and Mueller (15) reported similar large specimens from the doré and it is possible that they may represent a distinct subspecies.

Family Gorgoderidae

Genus *Phyllodistomum* Braun, 1899

As far as can be determined, up to the present time seven species of *Phyllodistomum* have been reported from American fishes. During the present survey, a species of *Phyllodistomum* was recovered from the urinary tubules of *Catostomus commersonii* and upon careful examination it proved to be heretofore undescribed. The name *P. lysteri* is proposed for this species in honour of the late L. L. Lyster by whom the first specimens of this species were collected.



Figs. 11, 12, 13. *Phyllodistomum lysteri, ventral view.*

Phyllodistomum lysteri sp. nov.

(Figs. 11, 12, and 13)

Host: *Catostomus commersonii* (common sucker)

The body of this species is flattened and made up of a narrow anterior region and a broad posterior part; the approximate ratio of the anterior to the posterior part of the body is as 1 : 2. The posterior end of the body is obtusely pointed and may show a small notch. The cuticula is smooth, and the body margins do not show any crinkling. Specimens range from

1.75 to 3.2 mm. in length by 0.5 to 1.0 mm. in width. The oral sucker is situated at the junction of the narrow and wider portions of the body; it may be the same size or very slightly larger than the oral sucker, measuring from 0.20 to 0.26 mm. in diameter. There is no pharynx. The oesophagus is approximately the same length as the oral sucker; it divides into two broad intestinal crura which extend to within a short distance of the posterior tip of the body. There are two large testes with irregularly lobed margins, situated in the middle third of the body. They are obliquely arranged with the left testis usually more anterior than the right, although this arrangement may be reversed. The lobed ovary is about one-third the size of the testes and is situated immediately anterior of the more posterior testis. The uterus fills the posterior part of the body between the intestinal crura, finally leading to the genital pore which is situated on the mid-line, a short distance above the acetabulum. There are two solid, lobed, vitellarian bodies, lying between the acetabulum and the genitalia, in tandem arrangement. The uterus contains what appear to be two types of eggs. One is a typical egg with an oval outline and a well developed egg shell, measuring 0.031 to 0.036 mm. by 0.018 to 0.021 mm. The other is spherical in outline and does not show a well developed egg shell. It is larger than the more typical egg measuring 0.032 to 0.052 mm. in diameter, and stains readily with alum carmine. The uterus of every mature specimen shows these two types of eggs.

Phyllodistomum lysteri is readily distinguishable from *P. pearsei*, *P. carolini*, *P. staffordi*, *P. lacustri*, and *P. fausti* by the fact that the oral and ventral suckers are not obviously different in size in this species, while they do show obvious difference in size in the last five species. It is further differentiated from *P. fausti* by not having the post-acetabular body widest in the middle and by the space between the testis, from *P. staffordi* and *P. carolini* by not having the post-acetabular part of the body discoidal, and from *P. lacustri* by having a smooth cuticula. It most closely resembles *P. superbum* and *P. folium*. However, it is distinguished from these two species by the more anterior position of its gonads and by the ratio of the pre- to the post-acetabular regions of the body which in *P. lysteri* is about 1 : 2, whereas in the *P. superbum* and *P. folium* it is 1 : 1.25. Furthermore it can be differentiated from *P. folium* by its larger size, and from *P. superbum* by the fact that the body margins are not crinkled.

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COLOUR OF MEAT

III. AN IMPROVED COLOUR COMPARATOR FOR SOLIDS[1]

By C. A. Winkler,[2] W. H. Cook,[3] and E. A. Rooke[4]

Abstract

A photoelectric colour comparator, previously described (Can. J. Research, D, 17: 1-7. 1939) has been improved to permit greater precision and more rapid operation. Measurements on meat indicate that the degree of precision attainable with the new comparator is determined primarily by sampling, rather than instrumental, errors.

Introduction

An objective colour comparator suitable for estimating the colour of solids was described in the first paper of this series (1). The results obtained with this instrument in subsequent investigations on the colour (2, 4) and colour stability of bacon (3, 5) led to the construction of an improved model. Enquiries requesting a more detailed description and other information regarding the earlier apparatus prompted the preparation of this article describing the new instrument.

The new apparatus, like the old, is based on the measurement of the amount of each main component of white light, namely blue, green, and red, scattered by the test sample and expressed as a percentage of that scattered by a standard white surface. Such measurements naturally yield less information than those made with a spectrophotometer, but have the advantage that they can be made more rapidly. This feature enables measurements to be made on labile materials without appreciable change, and facilitates making a sufficient number of measurements to estimate the variability present in such biological materials as meat. The equipment was originally designed to place the subjective visual estimates of colour and colour stability on an objective basis. Subsequent papers (3, 4, 5) have shown that not only was the older instrument capable of detecting significant differences in the colour of different samples, but it was also possible, by statistical studies of a large number of measurements, to relate the scatter of the individual colour components to the composition of the bacon with respect to certain constituents.

Description of Apparatus

The new instrument is fundamentally the same as the earlier model. The most important changes are: baffle plates to minimize internal reflections; diaphragm for controlling light intensity, thus enabling the light source to be

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operated at fixed voltage and avoiding major change in colour quality; a more sensitive photo-cell, and a more sensitive galvanometer. Mechanically the apparatus was much improved, permitting greater accuracy and more rapid operation.

A photograph of the apparatus is shown in Fig. 1 and a diagrammatic sketch in Fig. 2. It is impossible to give complete details of construction in this article but the information contained in the figures and the following brief description are considered adequate to permit the construction of a comparable instrument wherever the necessary facilities are available.

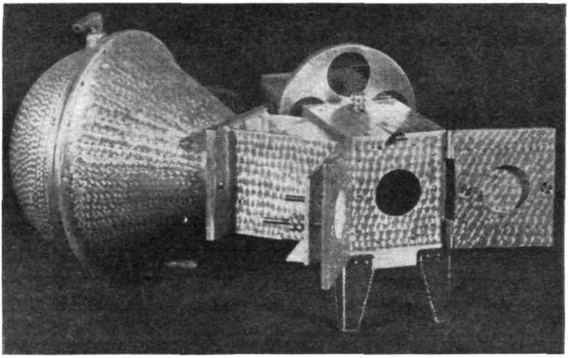

FIG. 1. *Colour comparator showing general arrangement, lamp housing, reference and test sample holders, and selective filter holder.*

The two sections of the lamp housing, A, and the adapter section, K, are dural castings with drilled and tapped flanges for attachment. The remainder of the apparatus is constructed from flat or angle aluminium fastened with screws to facilitate assembly.

The two sections of the lamp housing are water jacketed, and a continuous circulation of cooling water is maintained during operation. The hemispherical portion at the back is polished on the inside. A semi-circular opening is provided between the two halves of the lamp housing to accommodate a No. 2 photo-flood bulb. The walls of this portion of the housing are thicker and shaped to permit the attachment of an aluminium shield, supporting the lamp socket, to the housing with screws. As the photo-flood bulb must be screwed into the socket from the inside, the two sections of the housing must be separated when the bulb is changed, but as this is seldom necessary it is not a troublesome operation.

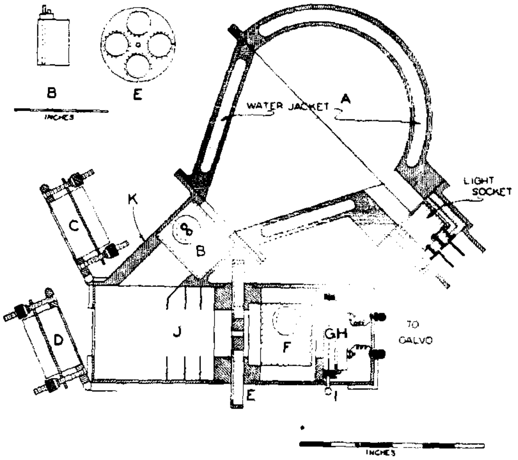

FIG. 2. *Diagrammatic sketch of colour comparator (plan view).*

The adapter section, K, is of rectangular cross section and flanged for attachment to the lamp housing. The end furthest removed from the housing is shaped to permit attachment to the metering portion at an angle of 45°. An oval opening, 1.5 in. high and 1.06 in. wide, is provided through the centre of this block, to allow the incident light at an angle of 45° to fall uniformly on the 1½ in. diameter circular opening containing the test sample. A rectangular recess, open at the top, is provided at right angles to the path of the light to accommodate the water cell, B, for removing part of the infra-red radiation from the incident light beam. This cell may be glass but a plastic (plexiglass) has been found more convenient. The cap for this cell is provided with two tubes for the circulation of cooling water (tap) during the course of the measurements.

The reference standard and test sample holders, C and D, consist of removable doors hinged on opposite sides of the rectangular part of the instrument. Each door consists of two plates arranged to hold the sample between them by clamping. The outer plate is solid, lightproof, and moves back or forth on the clamping screws. The inner plate is fixed and has a centrally drilled hole 1½ in. in diameter to expose the material. A similarly drilled plate member, darkened on the inside, forms the back of the instrument. The test sample is usually placed on an aluminium plate, or in a short cylinder with a locating pin if the exact test position is imoortant, and covered with

a clean glass slide before clamping in the holder. A similarly prepared cell containing magnesium carbonate is used as a reference standard.

Light scattered by the sample passes the baffles, *J*, and falls on a glass filter held in a rotating stainless steel holder, *E*, fitted in a light-tight manner into a slot in an aluminium block. Stainless steel was found to produce less friction and stand up better to the wear imposed on this member than an aluminium holder. Following the selective filters is a cell containing a 1% copper sulphate solution. This cell is identical with the water cell except that it has an ordinary screw cap since openings for water circulation are unnecessary. The iris diaphragm, *G*, is placed immediately in front of the photo-cell, *H*, and is adjustable by a short lever, *I*, at the side of the instrument. The centre inner surface between the sample holders and the photo-cell must be light-tight and finished with a flat black paint.

Accessory Equipment

The following accessory parts, obtainable from the stated sources, have been found suitable after considerable experimentation.

Plexiglass cells—made to dimensions and specifications by Stricker-Brunhuber Corp., New York, New York.

Selective filters—

Blue (400–500) Jena BG12, or Corning Signal Blue No. 556.

Green (500–600) Wratten W58, or a combination of Corning Light Theatre Blue No. 502 and H. R. Noviol No. 352.

Red (600–750) Jena OG3 or Corning H. R. Lantern Red No. 244.

Iris diaphragm—Leitz catalogue No. 8503.

Photo-cell—Visitron F-2A. G.-M. Laboratories Inc., Chicago.

Galvanometer—Box type Catalogue No. 4625, Rubicon Co., Philadelphia.

Transformers—The Variac transformer, General Radio Co., Cambridge, Mass., used for controlling the light source is generally unnecessary if an iris diaphragm is used, but may be required in special circumstances. Where line voltage is subject to some variation a constant voltage transformer will improve the accuracy and facilitate rapid operation. Suitable types are available from the Sola Electric Co., Chicago.

Operation

The manipulation of the new machine is simple and rapid. The filter holder is rotated until the desired filter is in position, the standard white holder swung into place, and the galvanometer deflection adjusted to the desired value with the diaphragm. The standard white holder is then swung out, the test sample swung into position and the galvanometer deflection observed. This sequence of operations can be performed in less than a minute for each colour filter used. In fact, with reasonably constant voltage, the three colour components can be measured in two minutes, including the

time required to place the test piece in the holder and remove it. This is a much more rapid rate than attainable with the older instrument and greatly facilitates the measurement of colour in routine operations such as process control. The apparatus is not only suitable for measuring the colour of such solids as meat, but has also been applied to ground materials, such as flour.

Accuracy

The accuracy of the new model can be compared with that of the old model, or with the error of sampling such biological materials as meat, from the results presented in Table I. The mean values and the standard errors of a single measurement were computed from duplicate measurements on some 30 to 40 samples picked at random from routine observations. The measurements on bacon with the old and new models were not made on the same samples and this may account for a portion of the difference between the mean values obtained with the two instruments. The greater part of this difference, however, is attributable to the leakage of light in the older model. The "dark" constant obtained with a standard black, as a test sample, was about 22% of that obtained from the standard white in the earlier model, and only 4% in the new model. This reduction in the dark constant was brought about largely by the insertion of the baffle plates, *J*, in the new instrument. The difference of 18% accounts for most of the difference between the corresponding means reported in Table 1.

TABLE I

PRECISION OF NEW COLOUR COMPARATOR AS COMPARED WITH THAT OF OLDER INSTRUMENT AND SAMPLING ERROR

Colour component	Old instrument		New instrument				
	Bacon only		Bacon		Pork		
	Mean	Std. error (instrument)	Mean	Std. error (instrument)	Mean	Std. error (sampling)	Std. error (instrument)
Initial colour							
Red	43.2	1.41	25.4	1.00	25.7	1.48	1.13
Green	31.1	0.87	14.5	0.45	16.2	1.12	0.77
Blue	28.3	0.61	11.5	0.36	11.9	0.74	0.64
Brightness					53.9	3.37	2.29
Colour stability*							
Red	5.35	1.30	4.03	0.60	2.19	0.43	1.04
Green	3.66	1.03	2.70	0.50	0.77	0.53	0.92
Blue	2.57	0.89	2.50	0.40	0.87	0.37	0.63
Brightness					3.83	1.33	2.22

**Mean change of scatter, usually a decrease from the initial value.*

The standard error of the instrument was computed by statistical methods *from the difference between duplicate observations.* The two values were obtained by cutting a piece of meat and making a single measurement on each of the two surfaces so produced. This practice tends to exaggerate the instrumental error by including the error of subsampling the test surface, and duplicate measurements on the same surface can be checked within narrower limits. The results of measurements of both the initial colour and colour stability of bacon show that the standard error of the new instrument is about half of that observed on the old instrument.

When the standard error of the initial colour measurement is expressed as a percentage of the mean for the corresponding colour component, the accuracy varies from about ± 2 to 4%. This may appear to represent satisfactory precision, but it must be remembered that a difference of 10 to 20% in scatter represents the difference between a satisfactory and wholly unacceptable colour as judged by visual standards. In these circumstances it might appear that a still higher instrumental precision would be desirable for making fine distinctions, without the need for tedious replication.

In connection with an investigation into the storage of pork, provision was made for estimating both the sampling and instrumental errors by making duplicate measurements on duplicate pieces of pork taken from the same part of the same carcass and treated identically throughout. An *analysis of variance* showed that the combined sampling and instrumental errors were significantly greater than the instrumental error alone. It appears therefore that the sampling error is the source of variation limiting the accuracy of initial colour measurements and that little, if any, increase in precision would be accomplished by further refinements in the instrument.

These results were analyzed further with the object of determining the *method of measurement capable of yielding the greatest precision for* a limited number of observations. The last two columns show the standard error of sampling and instrumental measurements independently, on a single observation basis. The sampling error for the initial colour estimations is in all instances larger than the instrumental error. It is therefore evident that four single instrumental observations on four separate samples taken from the test material would yield a more accurate estimate of the true colour than duplicate instrumental readings on two samples. In other words, the instrumental error is not the factor limiting the accuracy of colour measurements on meat.

A somewhat different situation exists with respect to the error of estimating the change in colour during colour stability measurements (lower section, Table I). Although the standard instrumental error is of the same order as that for the initial colour measurement, the change in colour scatter is much smaller than the original scatter and is consequently estimated with much less precision on a percentage basis. As can be seen from Table I, the sampling error of colour stability measurements is less than the instru-

mental error. In these circumstances precise estimates of colour stability can be obtained with the present equipment only by adequate replication.

From the standpoint of practical colour measurements on a particular material, meat, flour, etc., the most important factor is to select colour filters that will yield the maximum information on colour quality for the material in question. In this respect the new instrument is the same as the old, since the filters separating the three broad colour bands are the same. Spectroscopic studies of meat are now under way with the object of determining the practicability of using more selective filters. Should such a modification be desirable, it is possible that the use of extremely narrow colour bands might require more sensitive measuring equipment.

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STUDIES ON *OESTRUS OVIS* L.[1]

By A. Murray Fallis[2]

Abstract

The incidence of infection of *Oestrus ovis* larvae in lambs has been determined. Flies were obtained from larvae which pupated in the laboratory. The three larval stages of the fly were studied and characteristic features illustrated. Information relative to the rate of growth of the larvae was obtained from a routine examination of infected animals and experimental infection of lambs. The effect of the parasites on these experimentally infected animals was noted.

Introduction

The investigation was concerned with a study of the habits and development of the parasitic stages of *Oestrus ovis*. The incidence of infection was determined from an examination of sheep and lambs, one year old and under, received at a local abattoir from scattered points in Ontario and Western Canada. The rate of development was studied by the routine examination of slaughtered animals throughout the year and by experimental infection of parasite-free lambs.

Incidence of Infection

Of 698 animals examined, 50% were infected with larval stages of the parasite. A higher incidence would be obtained if the total included only the older animals, for over 90% of the lambs examined from August to May were infected.

Length of Life of Flies

Flies (Fig. 1) were reared from larvae which pupated in sand in the laboratory. Twelve flies were obtained in this way between May 2 and September 24, which are the first and last dates of emergence. Eight of the flies kept at room temperature lived an average of 16 days, one living for 28 days and two for 10 days only. This may be significant, for if flies remain alive and active for two weeks in their natural environment, a relatively small population might be capable of producing a high incidence of infection.

Mitchell and Cobbett (2) demonstrated that small, first-stage larvae could be expelled from female flies by pressing their abdomens. Hadwen has informed the writer that he used the same method to obtain larvae of a related fly, *Cephenomyia nasalis*. It seems likely, therefore, that *Oestrus ovis* is viviparous although Seguy (5) claims it is oviparous as well.

Larval Stages

The smallest larvae found measured 0.8 to 1.0 mm. in length following fixation in hot alcohol, whereas the largest measured 24 mm. The former were taken on September 2 and November 25 respectively, the latter on

[1] *Manuscript received August 14, 1940.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Ont.
[2] *Research Fellow.*

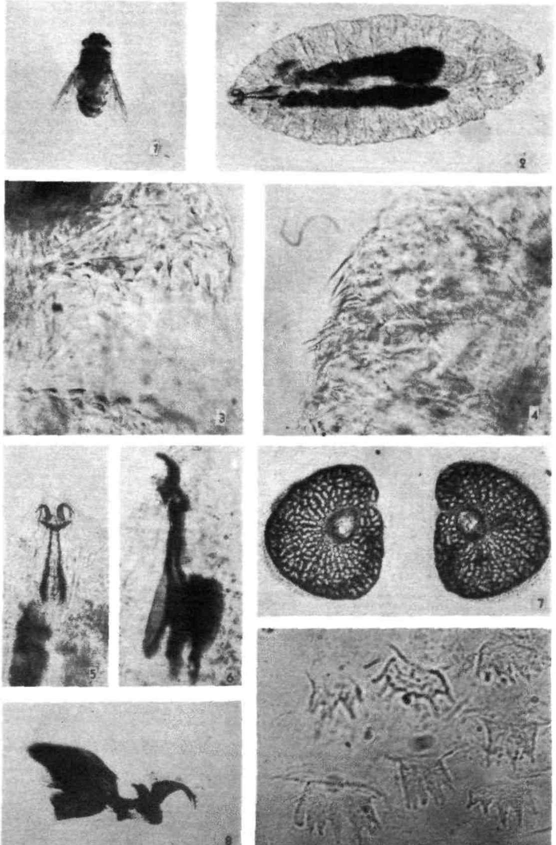

FIG. 1. *Adult fly, Oestrus ovis.* × 1½.
FIG. 2. *Ventral view of first instar larva. Note row of curved spines at posterior.* × 40.
FIG. 3. *Typical spines on ventral surface of segments of first-stage larva* × 300.
FIG. 4. *Typical spines at ventro-lateral margins of segments of first-stage. larva.* × 300.
FIG. 5. *Ventral view of pharyngeal sclerites and oral hooks of first-stage larva.* × 80.
FIG. 6. *Lateral view of pharyngeal sclerites and oral hooks of first-stage larva.* × 400.
FIG. 7. *Posterior spiracles of second-stage larva.* × 80.
FIG. 8. *Lateral view of pharyngeal sclerites and oral hooks of second-stage larva.* × 40.
FIG. 9. *Typical spinous plates on the ventral surface of the middle segments of second-stage arva.* × 400.

All the photographs were made from untouched negatives.

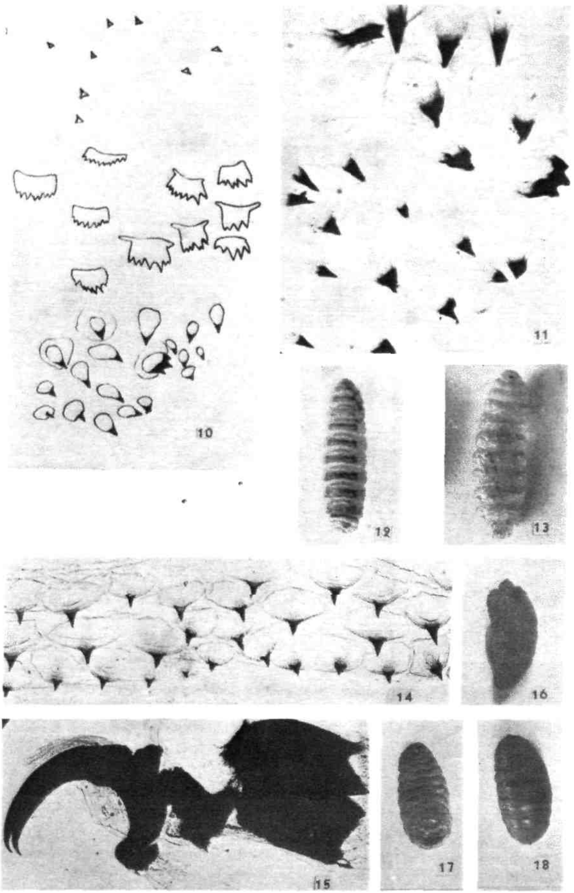

FIG. 10. *Outline drawings of typical spines on ventral surface of second-stage larva. Those on the anterior segment are shown in the upper part of the figure, those on Segments 2 to 10 in the middle part of the diagram, and those on Segment 11 in the lower part of the figure.* × 400.

FIG. 11. *Photomicrograph of typical spines on ventral surface of 11th segment of second-stage larva.* × *400.*

FIG. 12. *Dorsal view of third-stage larva.* × *1½.*

FIG. 13. *Ventral view of third-stage larva.* × *1½.*

FIG. 14. *Typical spines on ventral surface of third-stage larva. There are eleven rows of these.* × *40.*

FIG. 15. *Lateral view of pharnygeal sclerites and oral hooks of third-stage larva.* × *40.*

FIG. 16. *Lateral view of pupa.* × *1½.*

FIG. 17. *Ventral view of pupa.* × *1½.*

FIG. 18. *Dorsal view of pupa.* × *1½.*

All the photographs were made from untouched negatives.

March 16. First-stage larvae (Fig. 2) attained a maximum length of 5.0 mm. Characteristic features of this instar are the transverse rows of spines on the ventral surface of the segments (Fig. 3), the longer spines on the latero-ventral margins (Fig. 4), the row of curved spines on the posterior segments (Fig. 2), and the size and structure of the pharyngeal sclerites (Figs. 5 and 6).

Second-stage larvae measured 3.0 to 14.5 mm. The posterior spiracles of this stage are very apparent (Fig. 7). The pharyngeal sclerites and oral hooks are larger and have undergone some change in form (Fig. 8) from those of the previous stage. According to Portchinsky (3), quoted by Rodhain and Bequaert (4), the first-stage larvae, after penetrating to the sinuses, lose their spines and change to the second-stage. This he considers is a state of rest in which they pass the winter. It is evident from the present studies, however, that winter may be passed in the first-stage as well. Moreover, second-stage larvae are not devoid of spines (Figs. 9, 10, and 11), although they differ from those on first instar larvae. The most anterior and posterior rows of spines have a single spinous process (Figs. 10 and 11), whereas those on the intervening segments have the form of small plates terminating in a serrated edge (Figs. 9 and 10).

Third-stage larvae measured 9.5 to 24.0 mm. in length (Figs. 12 and 13). The eleven rows of spines on the ventral surface (Fig. 14) are prominent and all have a similar shape. The posterior spiracles are larger and more heavily chitinized than those of the second instar larvae. The pharyngeal sclerites are also larger and show further modification (Fig. 15).

Many of the lambs examined were harbouring all three stages of the parasite. This confirms an observation made almost 150 years ago by Clark (1) who wrote ". . . quite young and full-grown larvae may be found in the sinuses at the same time."

Rate of Development of Larvae

The rate of development of the larvae varies within wide limits. Spring lambs in Ontario are probably seldom exposed to the parasite before the beginning of May. No flies were taken in the field at this time but one was reared in the laboratory on May 2. On July 29 a larva, which formed a pupa from which a fly emerged, was recovered from the sinus of a lamb autopsied on this date. Thus it would seem the parasite cycle in spring lambs may require about three months in early summer. This confirms the work of Mitchell and Cobbett (2) who found the complete development of the parasitic stage in spring lambs in Texas and New Mexico required 2½ to 3½ months.

A study of natural as well as artificial infections indicates that development may not always be so rapid. This was shown by the presence of larvae less than 2 mm. long in the lambs examined throughout the fall and winter months when the temperature was such as to preclude the possibility of recent infection. On September 16, for example, 97% of 46 larvae obtained from five lambs examined were less than 5 mm. in length. Excluding one larva

which was 12 mm. long, the average length for the remaining 45 was 1.9 mm. On December 16, if we exclude one larva of 7.5 mm. from 75 obtained from five animals, the average for the remainder, which were all less than 3 mm., was 1.8 mm. On March 16, 43 larvae were recovered from two lambs. Three of these measured 15.3, 7.8, and 5.6 mm., respectively. The remainder were all less than 4 mm. in length, the average being 2.2 mm. A summary of the infection found in lambs at different times of the year is given in Table I.

TABLE I

—	Total number of larvae	Percentage of larvae in different size groups			
		0–5 mm.	6–10 mm.	11–15 mm.	Over 15 mm.
March	74	10.8	46.0	10.8	32.4
April	81	60.5	14.6	12.2	12.2
May	62	43.5	24.2	22.6	9.7
June		No parasites found in 50 spring lambs examined			
July	93	75.4	7.5	14.0	3.2
August	275	77.1	4.0	9.1	9.8
September	169	100.0–	+	0	0
October	89	97.5	2.5	0	0
November	380	98.6	1.4	0	0
December	209	98.5	1.5	0	0
January	62	96.7	3.3	0	0
February	62	96.7	3.3	0	0
March	93	94.6	3.2	1.7	1.7
April	64	78.0	7.8	11.7	3.0
May	50	54.0	16.0	20.0	10.0

The large number of first-stage larvae and the small number of third-stage larvae found from September to February is apparent. It is also seen that the number of second- and third-stage larvae recovered is much smaller than the number of first-stage larvae. The majority of the small larvae are found in the nares and many of them are probably sneezed out, whereas the larger larvae escape as they are usually found in the sinuses. Many first-stage larvae would be overlooked if the sinuses only were examined.

It was thought that the environmental temperature of the host might affect the rate of growth of the larvae. Three infected lambs were moved from an outdoor, midwinter temperature to a heated building, other infected lambs were left outdoors. One of the three lambs was left as a control. The second was given additional infection by transferring larvae less than 2 mm. long into its nostrils the same day as they were removed from infected animals. The third was given additional infection weekly by transferring small larvae to it. The first two lambs were slaughtered five weeks after being brought indoors and the third five weeks later. Control animals from outside were examined at the same time. The results are given in Table II.

An additional experiment was carried out in which two spring lambs, one month old, were infected by transferring larvae less than 2 mm. long to their nostrils. The lambs were kept in a heated building. Lamb No. 1 died

TABLE II

—	Time between infection and slaughter, in weeks	Number of larvae recovered	Maximum size, mm.	Minimum size, mm.	Average size, mm.
Lamb No. 1 (control)	5	5	2.5	2.2	2.3
Lamb No. 2	5	8	2.5	2.2	2.3
Outdoor control to No. 2	?	26	7.5	1.9	2.9
Lamb No. 3	10	50	18.0	1.5	5.0
Outdoor control to No. 3	?	15	5.6	1.9	2.5
Outdoor control to No. 3	?	28	15.3	1.8	2.4

of pneumonia eight weeks after being infected. It was autopsied, but not immediately following death so that some larvae may have escaped from it. Lamb No. 2 was autopsied five weeks after being infected. The results are indicated in Table III.

TABLE III

—	Time between infection and slaughter, weeks	Number of larvae recovered	Maximum size, mm.	Minimum size, mm.	Average size, mm.
Lamb No. 1	8	10	7.7	2.1	4.9
Outdoor control to lamb No. 1	?	17	21.0	1.9	11.1
Lamb No. 2	5	14	17.0	1.8	6.8
Outdoor control to lamb No. 2	?	27	17.0	2.0	7.0

It appears from the above-described experiments, therefore, that an increase in the temperature of the host's environment does not necessarily cause an increment in the rate of growth of the larvae. The rate of growth would seem to be variable at different times of the year. All the larvae used in the above experiments were several weeks old when transferred to the experimental animals and yet when these lambs were slaughtered several weeks later small larvae were still present. On the other hand, it was seen from the routine examination of spring lambs in early summer that the parasites reach maturity in the course of 2 to 3 months.

It also appears from these studies that there may be at least two generations of the fly per year in this country. In view of this, it is surprising that Rodhain and Bequaert (4) report only one generation of adult flies per year in Europe. They also point out the cycle is more rapid in Africa and that sheep are probably infected at all times since they always found second as well as third-stage larvae in all the animals examined. In the present study 63% of the infected animals harboured larvae in two or more instars. Most of the animals which harboured one stage only were examined during the

winter months. This raises the question as to whether or not there are more than two generations of the fly per year in Africa.

Pupa

Pupae (Figs. 16, 17, and 18) were obtained by leaving mature larvae in dry sand at room temperature (approximately 20° C.). Metamorphosis was not successful in all pupae. The first larva which pupated was taken on April 5 and the last on August 12. The pupal period varied from 19 to 34 days with an average of 29 days in 12 specimens.

Effect of Larvae on Host

The larvae caused a definite irritation to young animals. This was especially apparent in those which were experimentally infected. They sneezed and rubbed their noses on the wall. The "snuffing" was continued throughout life but was not so marked after the first few days. About two weeks following infection there was a slight discharge of mucus from the nostril of each lamb. At the time of autopsy the sinuses of both lambs were partially filled with purulent material. The pus contained large numbers of eosinophile leucocytes. Large numbers of these cells were also apparent in the mucosa of one of the lambs. First-stage larvae were frequently found with red blood cells in their intestines, but, as it was only found in those that had been bathed in blood, it is not known whether they were ingested by the parasite from blood present in the nasal cavities following the slaughter of the animal.

Acknowledgments

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